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Growth, 1951, **15**, 141-145

THE RESORPTION OF RIBOFLAVIN BY YOUNG AND OLD CULTURES OF *Lactobacillus casei**

ISADORE ROY COHEN[1] AND OTTO RAHN[2]

Laboratory of Bacteriology, New York State College of Agriculture
Cornell University, Ithaca, New York

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INTRODUCTION

The changes taking place in vertebrates during ageing are so complicated and involve so many tissues with different types of metabolism that it seems rather impossible to decide what is cause and what is effect. A better starting point for a study of the chemistry of ageing can probably be obtained by using simpler organisms. We have gone to the extreme in this respect by studying the ageing of the simplest of all organisms, i.e., bacteria.

In a bacterial culture, the cells multiply at a constant rate as long as the environment remains constant. When food becomes scarce, or metabolic products accumulate, multiplication becomes slower, and finally ceases. This is not comparable to vertebrates reaching old age; it corresponds to vertebrates reaching the adult stage, for these bacterial cells are at the height of their vitality; their rate of metabolism (e.g., the amount of metabolic products formed per cell per hour) proves that energy is readily liberated. But this energy serves only to increase the temperature of the culture; it is not utilized for synthesis, for growth, or for reproduction as in the juvenile stage of the culture. The cells have become mature, or adult; they have lost the power of multiplication. This status equals that of the adult stage of vertebrates.

It is at this stage that bacterial cells undergo a change. They can no more make use of the type of vitamins which the young cells needed for their development. Some part of the cell mechanism has lost its adaptability.

*Condensation from a thesis offered to the School of Nutrition at Cornell University.

[1]Present address: Commercial Solvents Corporation, Research Department, Terre Haute, Indiana.

[2]Present address: Department of Bacteriology, Idaho State College, Pocatello, Idaho.

This stage of rapid liberation of energy which cannot be used for synthesis or reproduction, this analogy of the adult stage of animals, is followed by a decrease and final cessation of metabolism, by a loss of vitality and by a gradual death of the cells. It is this stage of general decline which corresponds to the ageing of animals.

This first paper discusses the difference in behavior between young and old cells of *Lactobacillus casei* to the vitamin B_2, riboflavin. The lactobacillus needs riboflavin, but cannot synthesize it, and it can grow and multiply only if this vitamin is already present in the culture medium. The following paper describes the fate of the same vitamin in cultures of different ages of the same lactobacillus.

General Plan

In the bacteriological assay of riboflavin by *Lactobacillus casei*, the culture with less vitamin produces less lactic acid, and less turbidity, i.e., fewer cells. Experiments by Charlotte Liemer (unpublished) had shown that the cultures with small amounts of riboflavin never reach the acidity obtained with larger amounts, even when the cultures had been incubated for many days. This inability of the cultures with little vitamin to reach the same level as those with an ample vitamin supply might possibly be due to a gradual decrease in vitamin content brought about by cell activity. Animals requiring vitamins for growth continue to need them not only for growth, but in their adult life also. The vitamins consumed by animals do not remain perpetually active; they lose their efficiency for the organism by being either eliminated or destroyed, and they must be replaced. It appeared possible that in bacteria as well, riboflavin might be gradually destroyed by the life process, after becoming first a part of the cell protoplasm. In this case, the bound vitamin, if not destroyed, should be found in the young cells of the culture, and should decrease in the old cells.

This theory was tested in two different ways. Initially, we tried to recover riboflavin by acid hydrolysis from cells grown with optimum levels of riboflavin. The recovered part was normal riboflavin and could be used for growth of cells in the microbiological assay. An attempt was also made to furnish additional vitamin to cultures that had aged with sub-optimal amounts of vitamin, in order to see whether its life functions show a recovery.

Recovery of Riboflavin from Cells

Preliminary experiments showed that 3-days old cells, hydrolyzed and assayed for riboflavin, contained some riboflavin. The experiments were carried out in parallel, the cells of one set being extracted with petroleum and ethyl ether to remove any traces of fat or fatty acids from the cells which might possibly interfere with the assays (1, 2, 3). No differences were ever observed; the extraction did not change the riboflavin content of the cells.

To show the recovery and possible utilization of the riboflavin of the old cells by new cells, bacteria were grown for 72 hours in 258 ml. medium containing 30 micrograms riboflavin per liter. The culture was centrifuged, the cells were washed with saline, acidified with 30 ml. of normal sulfuric acid, and autoclaved for 15 minutes at 15 pounds pressure. Half of this cell suspension was then extracted as described above. If this cell suspension contained all the riboflavin that was used to grow the cells, one ml. of cell suspension should contain 0.121 micrograms of the vitamins. After neutralization, these suspensions were assayed for their riboflavin content by the standard microbiological titrimetric method (4) (Table 1). The recoveries

TABLE 1
Recovery of Riboflavin from the Centrifuged Cells of a 72 Hours Old Culture of Lactobacillus Casei

Standard Series		Original Culture*		Assay Record		
μg. riboflavin added	ml. $N/10$ acid increase	ml. cell suspension used	μg. riboflavin needed for cell growth	ml. acid increase	corresponding riboflavin	% recovered
0.05	2.28	0.4	0.0484	1.68	0.05	——
0.10	3.93	0.8	0.0968	2.61	0.06	62%
0.15	5.93	1.2	0.1452	3.83	0.095	65%
0.20	7.73	1.6	0.1936	5.60	0.145	74%
0.25	8.83	2.0	2420	7.05	0.19	79%
0.30	9.68	2.4	2904	7.55	0.21	72%
0.35	10.08	3.2	3872	9.19	0.26	67%
		4.0	4840	10.37	0.35	——
					Average	70%

*0.121 μg. riboflavin were needed to produce the cells in 1 ml. of cell suspension.

from the cell suspension were 70% of the originally added riboflavin. No measurable riboflavin could be found in the supernatant culture medium or in the wash water. In another series, using cells that were 18 hours old, the same technique gave average riboflavin recoveries of 82%.

Riboflavin assays using the chemical method showed recoveries of 59% for 72-hour cells, 70% for 48-hour cells, and 80% for 24-hour cells. Since the chemical and microbiological methods agree, it seems fairly well established that more can be recovered from young than from old cells. Hence, it is possible to recover from young cells about 80% of the riboflavin added to their medium and this riboflavin can be utilized by new cells when the old cells are killed and hydrolyzed before being added to the riboflavin-free assay medium.

While in these experiments with bacteria, which are in agreement with animal experiences, the vitamin becomes an insoluble part of the cell for a considerable length of time, that is not always the case. With vitamin B_{12}, Chow *et al.* (1950) found 80% of the subcutaneously injected vitamin to be excreted in the urine within 24 hours and Barbee and Johnson (1951) observed that of the injected vitamin in B_{12}, 93% were excreted in the urine within 24 hours, and of the orally administered vitamin, 89% was excreted in the feces. Vitamin B_{12} does not seem to become incorporated in the cell protoplasm.

Effect of Riboflavin Additions to Growing Cultures

With an insufficient amount of riboflavin, the culture of the lactobacillus soon cease to grow and to ferment. An experiment was made to see if a later addition of riboflavin to such cultures would result in renewed reproduction and acid formation.

Two cultures were used, one with 10 μg. riboflavin per liter, and one with 20 μg., and each was divided into four batches. Of these, one batch received 10 μg. riboflavin when 19 hours old, one received the same amount when 72 hours old, and one received the amount at both times, while the fourth one, as control, received none. Their acid formations are shown in Table 2. The total amount of riboflavin in the culture receiving the largest addition was only 40 μg. per liter, which is less than the optimal amount. Therefore, the inability of cells to respond to the addition could not have been caused by saturation of the cells with the vitamin, but was due to their age interfering with the vitamin assimilation.

In both sets of experiments, whether started with 10 or 20 μg. riboflavin, the renewed addition of vitamin at 72 hours remained without effect. Apparently, cultures are no more able to utilize riboflavin when 3 days old. The same addition to the 19 hours old culture resulted in an increased abiblity to ferment, though the cultures which were started with 10 μg. and received another dose of 10 μg. after

TABLE 2
ACID FORMATION WITH REPEATED DOSES OF RIBOFLAVIN

Riboflavin, in micrograms per liter			ml. 01 *N* acid formed at						
Initial Amount	Addition at 19 hrs.	Addition at 72 hrs.	19 hrs.	36 hrs.	59 hrs.	72 hrs.	88 hrs.	132 hrs.	
10 μg.	0	0	1.33	1.85	2.25	2.53	2.85	3.07	3.23
10 "	10	0	1.38	2.30	3.37	3.78	4.07	4.49	4.68
10 "	0	10	1.43	1.95	2.38	2.53	2.78	3.09	3.18
10 "	10	10	1.31	2.30	3.30	3.73	4.10	4.29	4.43
20 "	0	0	2.33	5.00	6.30	6.48	6.55	6.99	6.98
20 "	10	0	2.18	5.25	7.30	7.53	7.75	8.09	7.98
20 "	0	10	1.88	4.55	5.65	5.98	6.10	6.54	6.68
20 "	10	10	2.13	5.50	6.90	7.38	7.45	7.89	7.93

19 hours, did not reach the level that had been obtained with an initial dose of 20 μg.

SUMMARY

Riboflavin has been recovered from cells of *Lactobacillus casei* by hydrolysis in quantities of 70% to 82% of the amount added to the medium. More vitamin can be recovered from young cells than from old cells. The cell-free culture medium contains no measurable quantities of riboflavin.

This recovered riboflavin can be used by other cells for normal growth and acid production.

After the cells have once reached the resting stage, even when grown with an insufficient amount of riboflavin, they are no more capable of using additional riboflavin. A new supply of vitamin did not rejuvenate the cells if they had become old prematurely on account of an insufficient vitamin supply.

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THE FATE OF RIBOFLAVIN IN THE AGEING OF BACTERIA[1]

OTTO RAHN AND BARBARA ISKE

Idaho State College, Pocatello, Idaho

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OBJECT OF THE STUDY

The experience with metazoa has shown that vitamins are not merely growth factors, but are also needed throughout the adult life of multicellular animals. Avitaminoses of adult animals are well known. This paper tries to answer the question to what extent the experience with multicellular organisms applies also to unicellular organisms. An analysis was made of cultures of *Lactobacillus casei* at different ages. This bacterium cannot multiply nor produce significant amounts of lactic acid unless riboflavin is present in the culture medium.

The development of an animal is in some respects comparable to that of a bacterial culture. A single cell divides repeatedly, rapidly at first, and later more slowly, until a certain final number of cells is reached. This maximal number is reached asymptotically in both cases. A "full-grown" bacterial culture which has reached the maximal number of cells, is in several respects comparable to an adult animal. Cell division has ceased (except for the replacement of dying cells) while metabolism continues for a while, at first at a constant rate, but with progressing age at a slower rate. Ultimately in bacterial cultures, the metabolism ceases completely when the metabolic products reach an inhibiting concentration whereas in animals, metabolic products are continuously excreted. At this stage, the analogy between metazoa and bacterial cultures ceases to be perfect.

Since adult animals continue to need vitamins although they do not grow any more, and since old animals do not contain more vitamins than young animals, the conclusion seems inevitable that the vitamins of the consumed food are "used up" by the life process, either destroyed or eliminated. The main object of these experiments was to find out whether bacterial cultures also "use up" vitamins.

[1]This investigation has been made possible by a Public Health Service Research Grant.

METHOD

The answer to these questions could be obtained by a simple experiment. A number of cultures of the same bacterium, differing only in age, had to be analyzed for the various criteria of age (number of viable cells, concentration of metabolic products, vitamin in the cells, vitamin in the culture medium).

In order not to be misled by the instability of riboflavin, all cultures as well as the controls were kept at room temperature in the same semi-dark room. All cultures as well as the controls were analyzed on the last day of the experiment. To accomplish this, the cultures were not all started on the same day. The 32 days old culture was held as sterile medium for 8 days, and after inoculation on the 8th day, stood beside the flasks with the sterile medium for 32 more days when it was analyzed. The 2 days old culture was held as sterile medium for 38 days. It was inoculated on the 38th day, and two days later it was analyzed together with the other cultures and with the controls. If any chemical or physical deterioration of the riboflavin had occurred during the 40 days of the experiment, it would have affected cultures and controls alike, and all results concerning the riboflavin content are directly comparable. The riboflavin content of the sterile medium, after standing for 40 days in the semi-dark room, together with all the cultures, was 182 micrograms per 100 ml. of medium.

The culture medium for our tests was the assay medium for riboflavin by the Difco Company, to which a known amount of riboflavin was added. 100 ml. portions of this were sterilized in six-ounce screw cap bottles and inoculated on different days with a pure culture of *Lactobacillus casei*. The growth of this bacterium is independent of oxygen. When the oldest cultures were 40 days old, all cultures were analyzed. Part of each culture was filtered through a Seitz filter to remove the bacteria, and these filtrates were also analyzed.

The riboflavin assay was made according to the directions of the Texas Bulletin. Turbidity measurements were impossible in testing these cultures which already contained large numbers of cells, and we relied entirely upon the titration of the produced acid. It was necessary, of course, to neutralize the original acidity of the cultures of various ages. Our procedure was the following:

Of each culture, 20 ml. were pipetted into a screw cap bottle, and were autoclaved to kill all cells and destroy their enzymes.

The sterile contents were neutralized with normal alkali and 2 drops of phenolphthalein as indicator. Distilled water was then added to bring the total volume of 20 ml. culture plus alkali plus water with each sample to 25 ml. These diluted and neutralized cultures were then used for the riboflavin assay by the standard method. The cell-free filtrates were treated in the same way, except that sterilization was omitted.

Results

Acidity of the Original Cultures. The titration of the medium corresponded to 0.13% acid, expressed as lactic. The 2 days old culture titrated to 0.8%, the older cultures averaged 0.95% total acidity, calculated as lactic. This was the maximal acidity that this culture could produce in this medium; even the 40 day old culture did not differ significantly from this average.

Viability of the Cells. The colony count on a nutrient-glucose-phosphate agar showed a gradual decline of viable cells similar to the one usually observed in ageing cultures. The decrease shows the "logarithmic" decrease commonly observed in death by old age which proves a constant death rate (see Table 1 and and Fig. 1).

TABLE 1

Age in days	Viable Cells: Colony counts per ml. of culture			Riboflavin: Micrograms per liter			Percentage distribution	
	Total counts	Percentage Surviving	Dead	Culture	Cells	Filtrate	In cells	In filtrate
2	900,000,000	100	0	177	171	6	97	3
4	760,000,000	84	16	165	159	6	97	3
6	255,000,000	28	72	159	157	2	99	1
7	380,000,000	42	58	161	157	4	98	2
11	158,000,000	18	82	167	163	4	98	2
13	44,000,000	4.9	95.1	130	119	11	91	9
16	88,000,000	9.8	90.2	155	139	16	89	11
18	71,700,000	7.9	92.1	131	123	8	94	6
21	49,200,000	5.5	94.5	118	94	24	80	20
25	10,200,000	1.1	98.9	120	98	22	82	18
32	28,400,000	3.2	96.8	130	91	39	70	30
40	8,500,000	0.9	99.1	128	82	46	64	36

Fate of Riboflavin. Fig. 2 and Table 1 relate the fate of riboflavin. The total amount of riboflavin in the culture decreased noticeably from 182 to 128 μg. per liter in 40 days. This deterioration of the riboflavin was not noticed in the sterile medium. The decrease of about 30% of the vitamin content in 40 days must therefore be

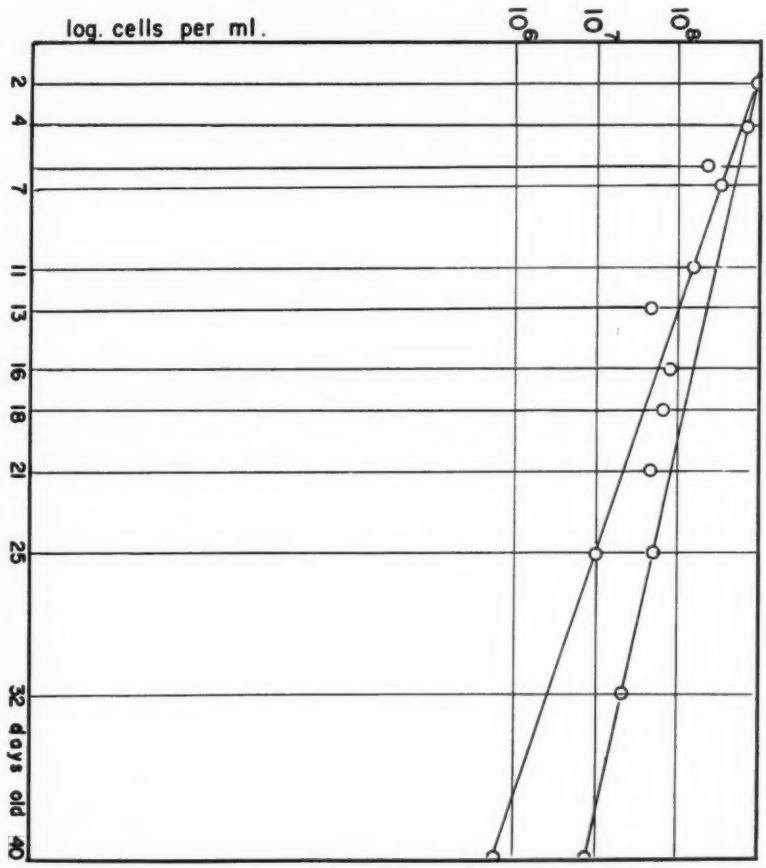

FIGURE 1.—Viable Cells in Young and Old Cultures.

accredited to the life activity of the lactobacillus. The importance of the life process in this destruction is confirmed by the evidence (Table 1) that of the total riboflavin, 97% became part of the bacterial cells, and only 3% remained as such in the culture medium. In this respect, thus, the cells of bacteria resemble the cells of multicellular animals. They require the vitamin for growth, i.e. for the production of new cells, and the adult cells gradually use up the

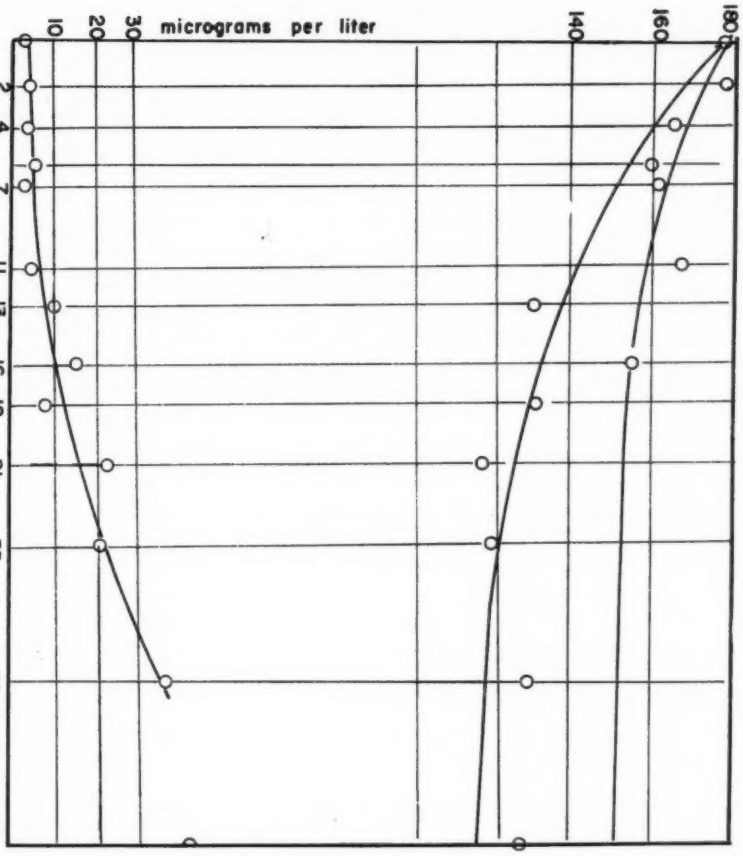

FIGURE 2.—Range of Riboflavin Destruction in Bacterial Cultures of Different Ages. Above: Riboflavin in Culture, upper and lower range. Below: Riboflavin in Cell-Free Filtrate.

vitamin and destroy it, i.e. change it to something that can no more serve as vitamin for the same species.

There is one important difference between the adult animal cells and the cells of a full-grown bacterial culture. The animal cells continue to require riboflavin, i.e. to utilize it, while the full-grown bacterial cells, according to Cohen and Rahn (see preceding paper) cannot

make use of this vitamin. This may be the reason why the cells of a full-grown bacterial culture die at a constant rate as soon as the culture has reached its peak of development while the adult animal maintains this peak for a certain length of time, several times as long as was needed to reach this peak.

Conclusions

The riboflavin content of a bacterial culture medium decreases when the riboflavin-requiring *Lactobacillus casei* is living in it. This does not happen with the sterile medium, and the decrease must be due to some life activity of the lactobacillus. The decrease in our case amounted to 29% of the total riboflavin content in 40 days, and the decrease does not start in later life, but is noticeable in all full-grown cultures. Whether any decrease takes place in growing cultures, was not investigated.

The riboflavin of the culture medium was almost completely incorporated in the cells. This will naturally depend upon the amount of riboflavin available. The statement is correct only as long as there is no excess of the vitamin. In our case, 180 μg. had been added per liter. Under the environmental conditions of our experiment, the 2 days old culture had reached the peak of vegetative development, 90 million cells per ml., and 97% of the riboflavin could be traced to the cells, while in the culture liquid, only 3% of the original vitamin amount were left. As the cultures became older, many cells died, and the riboflavin content of the liquid medium increased. It is probable that the dead cells released the vitamin into the culture medium. The death rate is more rapid than the riboflavin release, e.g.

at 4 days,	16% of the cells are dead,	3% of riboflavin is released
11 " ,	82%	2%
16 " ,	90%	11%
25 " ,	99%	19%
32 " ,	97%	30%
40 " ,	99%	36%

This proves that the vitamin of "dead" cells is not available at once; considerable time elapses before the vitamin of the "dead" cells can be found again in the culture liquid. That suggests a slow chemical breakdown of the vitamin-containing protoplasm, perhaps autolysis.

Summary

The growth of a bacterial culture and that of an animal show a similar behavior towards riboflavin: The young cells cannot fully develop without the vitamin. Insufficient vitamin results in insufficient development.

The adult animal continues to require vitamin. This proves that a certain amount of vitamin is being decomposed in the animal which must be replaced. Adult bacterial cultures also destroy vitamins. A culture of *Lactobacillus casei* lost 28% in 40 days.

The riboflavin of the culture medium becomes part of the cells and thus becomes insoluble. The cell-free filtrate from young cultures contains little or no riboflavin. But as the culture gets older, the cells die, and the riboflavin in the culture medium increases again. At 40 days, one-third of all riboflavin has been released again. However, as 99% of all cells at this time have lost their viability, the release of vitamin from the cells is not simultaneous with the loss of viabiltiy. There is no evidence that viable cells release vitamins.

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EFFECTS OF X-RAYS ON SNAILS, CRUSTACEA, AND ALGAE

KELSHAW BONHAM AND RALPH F. PALUMBO

Applied Fisheries Laboratory, University of Washington, Seattle, Washington

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INTRODUCTION

Early in the century studies of the effects of X-rays on biological material were conducted without evaluation of dosages. A little later, critical attention was given to dosages, but units of dosage and measuring devices were not yet standardized. Gajewskaja (1923) reported several morphological anomalies in *Artemia salina* following X-irradiation experiments conducted as a basis for possible comparison with the effects of naturally occurring radioactivity in the salt sea of Sebastopol. X-raying produced altered numbers of abdominal segments, change in heart length, eye deficiencies, sterility, and gill and setal variations which were classified as either hereditary or non-hereditary. Sonehara (1933) found that the degree of development attained by *Lymnaea* (*Radix*) eggs was inversely related to X-ray dosage. Conversion of the dosages to roentgens for the works of Gajewskaja and Sonehara is attempted in the discussion section of the present paper. Luyet (1934) working with the green alga *Stichococcus bacillaris* showed that the number of cell divisions after 6 days declined precipitously with an X-ray dose of only about one-sixth of the "lethal dose", and then gradually approached zero at about one-half of the "lethal dose".

It soon became common practice to express dosages in roentgen units rather than only in units of time. There was a trend away from survey types of studies toward analysis of the nature of the destructive actions of the rays, using precise physical measurements and carefully controlled biological materials. Because of the ease of culturing and the great numbers involved, microorganisms were the most appro-

This report is based upon work performed under Contract Number W-28-094-eng-33 with the Atomic Energy Commission.

priate subjects for these studies, among which may be mentioned the pioneer work of Crowther (1926) who studied the dosage-mortality relationship for *Colpidium*, and later, that of Ralston (1939) on *Dunaliella*. Probably as a consequence of this trend toward precision the literature is relatively devoid of information concerning invertebrates whose mass responses to X-rays seem to involve more experimental variability than is the case with microorganisms.

The present report is a summary of experiments that were undertaken with the objective of determining the lethal dosages, particularly the doses at which 50 per cent succumb (LD_{50}), for some common aquatic organisms. These data will serve as a background for possible future experiments with radioactive food chains, and will contribute to our knowledge of factors affecting the lethal actions of ionizing radiations.

Materials and Methods

Collections of the snail, *Radix japonica* (Jay) were obtained on October 24, 1948, and January 18, 1949, from the creek in Richland, Washington, through the courtesy of the staff of the Aquatic Biology Laboratory of the General Electric Company, Hanford Directed Operations, and were identified by Dr. Joseph P. E. Morrison of the United States National Museum. Samples of the common marine snail, *Thais lamellosa*, were obtained March 30, July 6, and October 15, 1948, at Meadow Point, Seattle. At the same locality collections were made on January 14 and March 19, 1948, of littoral marine amphipods, identified by Mr. Clarence R. Shoemaker of the United States National Museum as consisting primarily of *Calliopius laeviusculus* (Kröyer) and *Allorchestes angustus Dana*. Brine shrimp eggs, *Artemia salina* L., were collected from Leslie salt ponds in San Francisco Bay, February 1946 (*Artemia*-1 and -2), and February 195 (*Artemia*-3, -4, and -5), and donated by the Steinhart Aquarium through Mr. Robert P. Dempster. Fresh water algal cultures were provided to one of the writers (R. F. P.) by Dr. Margaret T. Dyar of the Botany Department, University of Washington, from the following sources: *Chlorella* and *Ankistrodesmus* from Seattle, *Chroococcus* and *Synechococcus* supplied to her by Dr. M. B. Allen, Stanford University.

For all but certain experiments with algae the X-raying was done with a Picker pulsating therapy generator (Cat. No. 1022) and a

Machlett FCX tube with tungsten target. The generator was described by the manufacturer as the two-valve Villard voltage doubling circuit type with a voltage wave shape similar to half-wave rectification but with a higher effective voltage. Peak kilovoltage (PKV) was 200 except where noted later for the second *Artemia* experiment, and the current was 20 MA. The ionization chamber was removed so that the effective filtration involved both the inherent filtration of the oil-immersed tube head equivalent to 0.25 mm. copper and 1 mm. aluminum, plus two metallic filters either 0.25 or 0.5 mm. copper and 1 mm. aluminum, making a total of either 0.50 or 0.75 mm. copper and 2 mm. aluminum.

Experiments with algae in August 1950 were conducted with a Picker Army field X-ray unit (soft X-rays) operating at 55 PKV and 15 MA to deliver 3.5 kr (a kiloroentgen is 1,000 r) per minute through an aluminum filter of 0.15 mm. thickness. Long exposures with this machine were intermittent, 12 minutes on and 4 minutes off, to allow the X-ray tube to cool. Exposures were determined with a Victoreen thimble chamber r-meter of either 25 r or 250 r capacity. Backscatter was negligible because wooden tables were used and the distance from the specimens to the concrete floor or lead ceiling was from 3 to 5 feet.

Table 1 gives information on some of the conditions pertaining to X-raying and culturing. Additional information not readily tabulated is given below. Invertebrate experiments are individually designated by appending the number of the experiment with that organism, i.e., *Thais*-3 where this is experiment number 7 in Table 1, while experiments with algae bear only their number in the entire series of the paper, i.e., *Chlorella* 28-30. This treatment is convenient because there is occasion to consider individual invertebrate experiments, but only groups of experiments with algae. *Amphipod* and *Algae* are italicized for clarity. In addition to the exposures listed in the dosage column, controls (0 r exposure) were involved in all experiments except as noted later for *Chlorella* 31-32.

Specimens of *Radix* were marked individually for identification with white india ink numerals on the largest whorl of the shell and covered with 2 coats of 2% "Formvar E" in ethylene dichloride. The sequence in which the doses were administered has a bearing upon the results in *Radix*-1. Of the 100 serially numbered snails the first eight were given 500 r; the next four, 0 r; the next eight received 1,000 r; the

Table 1. Experimental conditions of exposure to X-rays and culturing.

Experiment no.	Organism and stage X-rayed	Date of X-ray	Cm. to anode & hardness	Dose rate, r/m	Doses in kiloroentgens in sequence given	Length attained mm.	Numbers and distribution of specimens among containers	Degrees C, culturing	Food per container	Frequency census days	Date exp't. ended	Duration, days
1	Radix adult	Nov 12 1948	15 hard	500	0.5; 1; 2.5; 5; 10; 20; 40; 80.	12-21	18 control, 4 of each dose group in either of 2, 20-gal. aquaria.	13-16	little; algae on aquaria; 0.1 gm. wheat 3 times.	1-2	Apr 30 1949	169
2	Radix adult	Jan 18 1949	15	450	0.5; 1; 2.5; 20; 5; 10.	17-25	2 control, 1 of each dose group in each of 4, 3-gal. aquaria.	17-25	none	1-2	Mar 11 1949	52
3	Radix adult	Feb 1 1949	15 30	600 120	10; 3.1; 1; 0.3. 0.1.	11-23	5 of the same dose group in each of 6, 3-gal. aquaria.	16 (11-17)	lettuce; algal scum	1-2	Apr 15 1949	73
4	Radix eggs	Jan 29 1949	15	500	10; 1; 0.18.	1	71-111 eggs of the same dose in each of 4, 1-gal. jars.	15-17	lettuce; algal scum	1-2	Mar 8 1949	38
5	Thais adult	Apr 2 1948	16 25 33	400 160 100	10. 5. 2.5; 1.25; 0.5; 0.2.	40-57	45 control; 9 each group 200r-2.5kr; 2, 10kr; snails divided among 3, 20-gal. aquaria.	7-13	none	1-2	May 27 1948	55
6	Thais adult	July 6 1948	13 24	660 200	20; 10. 5.9.	42-63	17 control, 8, 5.9kr, 4, 10kr, 1, 20kr; 1, 20-gal. aquarium.	13 (8-21)	none	1-50	Aug 10 1950	765
7	Thais adult	Oct 20 1948	25 29	160 100	30; 10. 17.	36-50	16 control, 8 each dose group in 1, 20-gal. aquarium.	13 (8-21)	none	1-50	Jan 23 1950	460
8	Amphipod adult	Jan 15-16,'48	17	340	0.15; 0.9; 6.4; 16; 32; 82.	3-12	20 per 1-gal. jar: 6 control jars, 6 dose group jars.	7-12	bits of liver, meal, & Ulva every 7-20 days.	1-3	Apr 20 1948	96
9	Amphipod adult	Mar 9 1948	17	340	0.2; 0.5; 1.25; 5; 10.	8-11	30 per 1-gal jar: 6 control jars, 6 dose group jars.	7-12	liver twice; Ulva	1-3	Apr 28 1948	50
10	Artemia eggs	May 12 1949	7.5	2,100	100; 1.5.	1	240,000 eggs in each of 3, 1-gal. jars.	22	none	1	Jun 1 1949	20
11	Artemia eggs	Oct 20 1949	13 40	900 76	80; 8; 0.8; 0.08; 0.008.	1	240,000 eggs each of 12, 1-gal. jars; 2 series, dry and wet.	22	none	1	Nov 10 1949	20
12	Artemia eggs	Oct 11 1950	7.5	2,000	31; 10; 3.1.	1	200,000 eggs each, 12, 1-g jars; 3 series, 1 dry, 2 wet.	20-26	10 mg. floating algal scum, 8th day	1-2	Oct 24 1950	13
13	Artemia eggs	Feb 24 1951	7.5-15	470-2,240	71.8; 44.6; 29.8; 20.8; 15.1.	1	200,000 eggs each of 12, 1-gal. jars; 2 series, dry and wet.	21	none	1-2	Mar 2 1951	6
14	Artemia eggs	Mar 3 1951	7.5-15; all above, hard.	dry 440-2,160 wet 280-1,870	93; 57; 38; 26; 18.8. 80; 46; 28; 18.2; 12.1.	3	200,000 eggs in each of 12, 1-gal. jars; 2 series, dry and wet.	21	1-2 mg. yeast every 2-3 days	½-4	Mar 24 1951	21
15-19 20-21 22-24 25-27	Chlorella Ankistrodesmus Chroococcus Synechococcus	Aug 14 to Nov 29, 1950	3.3, soft; 7.5, hard.	3,500, soft; 2,000, hard.	100; 60; 50; 31; 10; 3.1; 1; 0.31.	-	1 ml. X-rayed in tubes; 8-12 tubes per exp't; diluted 1:10 for culturing and again when series became crowded.	25-28	liquid, nutrient media	1-7	Dec. 1950	7-54
28-30	Chlorella	Oct-Nov 1950	7.5, hard.	2,000	100; 60; 50; 31; 10; 3.1; 1; 0.31.	-	29 tubes; same or next day diluted 1:10, plated 50-600 cells, each.	25-28	nutrient agar	1-7	Nov 22 1950	14-25
31-32	Chlorella	Feb 19-21, '51	10, hard	1,460	35; 30; 26; 24; 20; 15; 12; 10; 6; 5; 3; 0.8; 0.4; 0.3.	-	2,000 or 2,800 cells per plate; X-rayed 4 areas on each of 4, 10-cm. Petri plates.	21	nutrient agar; Davis (1950) complete medium	once only	Feb 27 1951	6-7

next four 0 r; and so on up the series of exposures. Each exposure group and the following control group were divided in two and put into alternate aquaria so that the experiment was run in duplicate. Adult snails were X-rayed from above in glass dishes in air, and the eggs of *Radix*-4 were in sea water 4 mm. deep in an 18 x 5-mm. glass cup.

Aeration with an aquarium pump was used for *Radix* after the first 44 days of *Radix*-1. Air was released through carborundum releasers at first, but these were dispensed with in favor of open tubes. Tubes were J-shaped and of 2-mm. inside diameter, releasing air at the rate of about 100 cc./min. *Radix* jars underwent changes of water from 1 to 3 times during the course of each experiment; the pH of the water in the different jars varied from 7.2 to 8.0 on the one occasion when it was determined. Subdued room light prevailed. Censusing was usually done daily or even more frequently at first, but at greater intervals later in the experiments as the rate of change of results decreased. Motility was used as a criterion of viability in all animal experiments.

For *Radix, Thais* and *Amphipods* all individuals were observed at each census and living and dead were recorded. Individual specimens of *Thais* were marked with pencil, ink, or stenciled numbers. *Thais* tanks were kept full of Puget Sound water which was renewed once or twice during each experiment. The containers were kept in troughs of refrigerated water. Failure of the cooling mechanism accounted for short periods of high temperature accompanied by mortalities. Subdued room light prevailed. *Thais*-2 and -3 were aerated through straight tubes releasing near the bottoms of the tanks at the rate of about 500 cc. of air per minute. In *Thais*-1 there were 33 groups of egg cases attached to the glass near the tops of the aquaria on April 7, 1948, apparently laid by both irradiated and control snails. None hatched. In *Thais*-3 both a control and a snail exposed to 17 kr spawned on November 26, 1948, at a temperature of 12° C. and others spawned in late March 1949, at 11° C. Again there was no hatching. In *Thais*-2 the length of the 20 kr specimen (63 mm.) exceeded the next longest by 8 mm.

Amphipods were exposed in sea water 3 mm. deep in a syracuse watch glass of 5-cm. diameter. The culture jars were kept approximately two-thirds full of sea water from Puget Sound, without changing or renewal. They were partially immersed in troughs of cool

water. Subdued room light prevailed. In *Amphipod*-1 mating pairs and ovigerous females were recorded. For censusing, adult specimens were removed from the jars to a dish under the microscope using a syringe. In *Amphipod-2* a separate syringe and dish were used for each culture to avoid contamination. Those offspring removed incidentally along with the adults were counted in both experiments as an index of reproduction. Survivors at the end of the experiments numbered 1-9 in each of 7 jars of *Amphipod*-1, and 1-13 in 9 jars of *Amphipod*-2.

In all five *Artemia* experiments, aliquots of 200 mg. of eggs were used for each jar. The kind and amount of water in which the *Artemia* eggs of the wet series soaked before being X-rayed along with the number of hours of soaking in the four *Artemia* experiments concerned, were: -2, 5 ml. of tap water, 24 hours; -3, 2 ml. of sea water, 15 and 24 hours; -4, 1 ml. of sea water, 15 hours; and -5, 2 ml. of sea water, 10 hours. In *Artemia*-4, the 1 ml. of sea water in which each cupful of wet eggs started soaking, evaporated within about 15 hours so that the eggs were practically dry when X-rayed. In the dry series the eggs filled even the smallest containers to a depth of only 2 mm. For *Artemia*-2, -4, and -5 the dry series was X-rayed before the wet series. In *Artemia*-3 the three series were X-rayed at 7 a.m., 10 a.m., and 4 p.m., respectively. All were put into the culturing jars immediately after X-raying. Except in *Artemia*-3 as just indicated, the intervals between exposures were only the few seconds required to change containers. In *Artemia*-4, and -5, the 5 egg cups of a series were stacked in a paper cylinder over the inverted X-ray tube and irradiated simultaneously from below. The difference in dosage arose from the difference in distance from the target and from filtration by glass, eggs, water, and air at the successive levels. The 5th root of the difference in intensity between the top and bottom of the stack of 5 cups, as measured with the Victoreen r-meter, was extracted as the amount of reduction due to 1 cup of eggs and the dosages at the various levels were computed. It was assumed that the reduction effected by one cup of eggs influenced its own and succeeding cups of eggs.

In *Artemia-2*, trouble with the X-ray machine required reducing the kilovoltage to 185 for the wet series.

Artemia culture jars were kept from one-half to two-thirds full of salt water from Puget Sound without changing or renewal. Moderate

room light prevailed. The jars were cleared of shells and dead eggs by siphoning off the culture and rinsing the jar. The cultures were aerated through 2-mm. J-shaped, open tubes at the rate of about 100 cc. per minute.

Because of the large numbers of *Artemia* it was necessary in censusing to use only samples. They were obtained immediately after stirring the culture thoroughly with a spoon. Hatched larvae were counted from the sample dish as they were removed with a fine dropper. Early in some experiments more than one count per day was made, but usually only one. Some samples were preserved in 1.5 per cent formalin in sea water for growth determination. Horizontally oriented larvae were measured at first from photographs, and in later experiments, directly, using an eyepiece grid 10 units square. One of the central squares was subdivided into 25 small squares making it possible to measure directly to fifths of units, but usually the subdivided square was not used and tenths of units were estimated. The cuticle of the preserved *Artemia* larvae was usually elevated from the protoplasmic portion of the body. Lengths were measured to points half way between the elevated cuticle and the cellular portions of head and tail.

For *Algae* 15-30 a suspension of from 0.1 ml. to 1.0 ml. of fast growing algae was X-rayed in glass tubes. In the experiments with soft X-rays the cultures became as warm as 40° C. during exposure, but were cooled in ice water periodically. Controls were also warmed and cooled. After irradiation 10 ml. of nutrient solution were added and the culture was placed in a light box about 16 inches from a pair of 30-watt fluorescent tubes for continuous illumination. In some of the early experiments with algae, numbers of organisms per unit volume were determined using blood counting chambers as well as colorimetrically, but later, growth in liquid medium was evaluated only by measuring the light-stopping ability of the agitated culture using a photoelectric colorimeter (Klett) because the two methods produced results that did not differ significantly.

In experiments with *Chlorella* 28-30, after irradiation in 125-mm. test tubes, the cultures were diluted 1:10,000 or 1:1,000,000, plated out on nutrient agar cooled to 40° C., allowed to gel, and placed in the light box until ready for counting. The colonies which grew on the plate and were visible to the naked eye were counted. The average number of colonies on the control plates was considered to be 100%.

For *Chlorella* 31-32 the four dilution plates were made on nutrient agar about 1 mm. thick. To the bottoms were taped cellophane grids bearing india ink rulings at 5-mm. intervals. X-raying was done from below through a 25-mm. square hole in a shield of lead 2.4 mm. thick, shifting the plate over the hole between exposures in order to irradiate four areas on each plate. Leakage through the lead plate at the rate of about 10-15 r/min. gave the dosages of from 300 r to 800 r for the control areas. Because of the small area of exposure—about one square inch—the counting was done with a stereoscopic wide field binocular microscope when the colonies had attained a diameter of 20-100 micra (5-7 days). All colonies on each plate were counted. The cultures were exposed to bright room light throughout the day but not to direct sunlight.

Results

Radix

Figure 1 shows the mortality rates in *Radix*-1, -2, and -3 for the different irradiation groups and Figure 2 shows regression lines fitted to the same data. Because the mortality-time relationship is typically curvilinear as is suggested by the controls in *Radix*-1, these straight regression lines are of limited value but serve to show the trend of the observed points. *Radix*-1 provides most information because of the greater number of specimens and longer period of survival. *Radix*-2 and -3 show something of the variability encountered in different experiments. Inspection of Figure 2 for *Radix*-1 shows mortality rates greater than the control's in groups given 5,000 r or more and in the 500 r group. It is felt that the high mortality rate

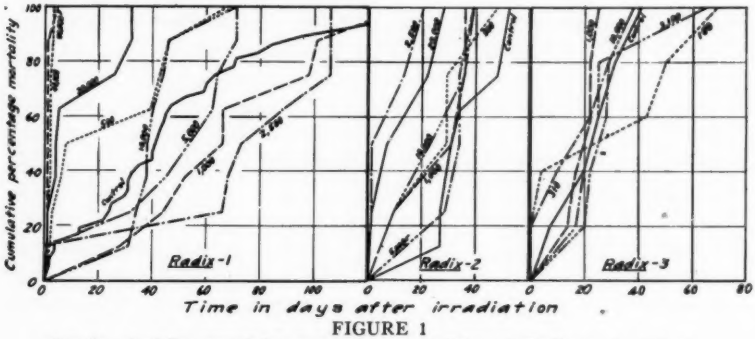

FIGURE 1
Graphs of daily cumulative percentage mortalities of *Radix*-1, -2, and -3.

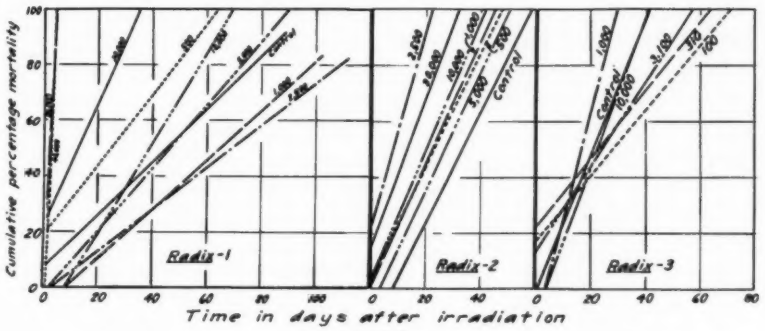

FIGURE 2

Regressions of cumulative percentage mortality upon time in *Radix*-1, -2, and -3, from data of Figure 1.

of the 500 r group (the first group irradiated) was caused by somewhat overdoing an attempt, at the time of picking up the specimens for serial numbering, not to leave less active specimens until last as is the natural tendency in sampling. Comparison of mortality rates (regression coefficient of mortality on time) by the method of Fisher (1946: 140) in the various irradiated groups with the control group revealed the following levels of probability that the differences could occur by chance alone:

500 r $\approx$ 0.01; 1,000 r » 0.05; 2,500 r » 0.05; 5,000 r $\approx$ 0.05;
10,000 r $\approx$ 0.001; 20,000 r $<$ 0.001; 40,000 r $<$ 0.001;
80,000 r $<$ 0.001.

Thus, aside from the 500 r group disregarded for reasons stated above, the 5,000 r dose was at the borderline of statistical significance, lower doses were below, and higher doses above.

In *Radix-2* the unexplained high mortality rate of the 2,500 r group complicates evaluation of effects in other groups. The regression coefficients differed non-significantly from the controls, in most cases far short of the 5 per cent level of probability. Only the 2,500 r group approached the 5 per cent level. If the 2,500 r group be disregarded as erratic from some extraneous cause, the mortalities in the 10,000 r and 20,000 r groups assume their expected positions of high mortality relative to the other doses. Of biometric interest might be the fact that the mortality *rates* are fairly uniform, while the displacements

of the lines from the controls are considerable. A better tool than comparison of regression coefficients is to be desired.

In *Radix*-3 extraneous conditions obviously increased the mortality rates of the control and 1,000 r groups. The 10,000 r group rate was greater than that of the other three lower dosage groups as would be expected.

Figure 3 combines information from the three sections of Figure 1 to show the relationship of dose to the time at which 50 per cent of

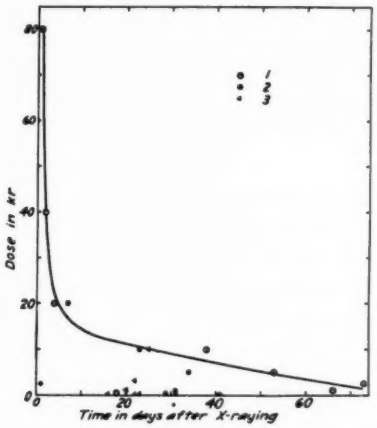

FIGURE 3

X-ray dose as a function of time (scaled from Figure 1) to attain 50 per cent mortality in *Radix*-1, -2, and -3. Curve drawn by inspection to the points of *Radix*-1 exclusive of 500 r and controls.

the snails subjected to that dose, succumbed, without compensating for control mortality. The coordinates were scaled from Figure 1 at the points where the curves crossed the 50 per cent level. The curve of Figure 3 was fitted by inspection to the points of *Radix*-1 disregarding the 0 r and 500 r groups. At 20,000 r or more 50 per cent of the snails died within 7 days; at 10,000 r, in from 23 to 38 days; and at 5,000 r, in the neighborhood of 50 days, but below this dosage level the relationship was erratic.

It was determined for *Radix*-1 and -2 that mortality was not significantly related to length of specimens.

The design of the *Radix*-3 experiment with only one dosage group to a container permitted observations of reproductive activity at the

different dosage levels. Egg-laying occurred in the groups with the lowest and highest mortality rates—100 r and 1,000 r—but not in the control, 310 r, 3,100 r, or 10,000 r groups. The control tank became murky with *Chlorella* which seemed to be detrimental. Clear water and brown scums of diatoms on the glass favored growth in the 1,000 r tank where one young snail grew from 4 to 12 mm. in length in one month.

In *Radix*-4 where the eggs were irradiated in the masses there was little difference in mortality between the controls and any of the irradiated groups. At the end of the experiment (23 days) the numbers of survivors in the four groups were: control, 24; 180 r, 50; 1,000 r, 38; and 10,000 r, 42. Figure 4 shows the percentages of the

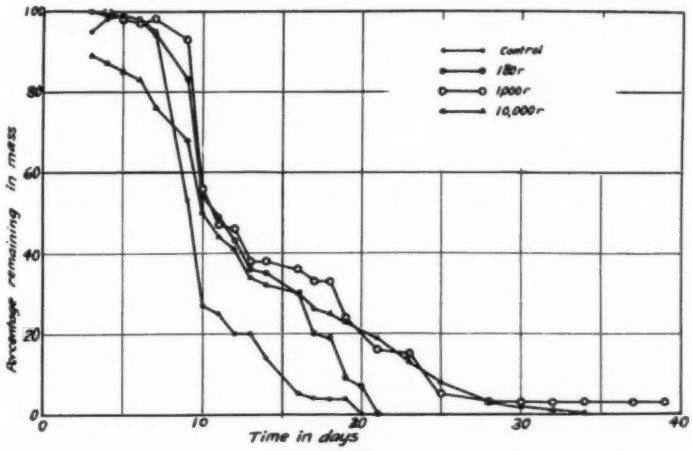

FIGURE 4

Abundance of *Radix*-4 eggs remaining in the masses expressed as percentage of original number, as a function of time after X-raying.

original numbers remaining in the masses as a function of time. There was a tendency for the embryos to develop more slowly and to remain in the egg masses longer after higher dosages. During the last week of the experiment the dead were preserved and numbers of hatched and unhatched respectively in the different groups were: control, 17 and 0; 180 r, 35 and 8; 1,000 r, 28 and 29; and 10,000 r, 2 and 28. A preponderance of unhatched larvae in the higher dosage groups prevailed similarly among living specimens. Size at one week after

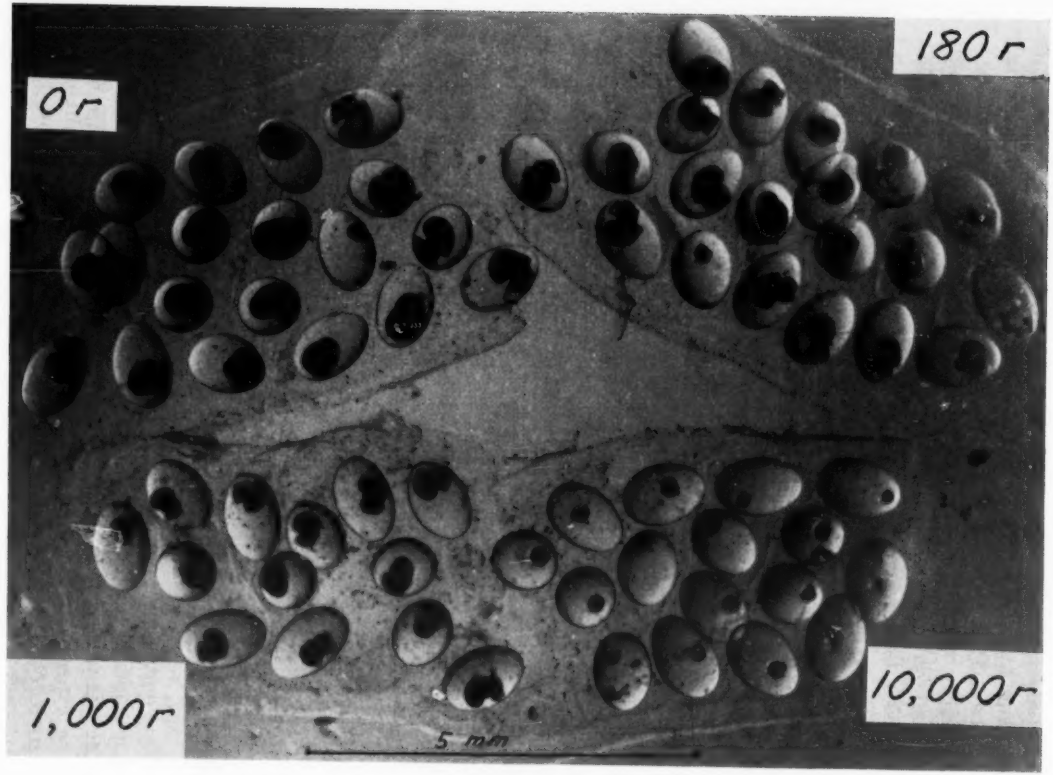

FIGURE 5
Photomicrograph of four portions of an egg mass of *Radix*-4 one week after being X-rayed with the indicated dose.

X-raying was determined on a single photograph (Figure 5) taken February 5, 1949, of samples of the earliest stage present (4 portions from a single egg mass) in the 4 irradiation groups with the results shown in Table 2. Recognizable embryos failed to form in the 10,000 r

TABLE 2

MEANS AND STANDARD ERRORS OF DIMENSIONS IN MICRA OF *Radix*-4 EGGS FROM A PHOTOMICROGRAPH TAKEN ONE WEEK AFTER X-RAYING. SAMPLE SIZE, 13-20 SPECIMENS.

Dose in r	Greatest dimension of larval shell	Least dimension of egg membrane
0	528 ± 12	723 ± 3.0
180	471 ± 10	743 ± 5.3
1,000	370 ± 8	713 ± 3.1
10,000	218 ± 8	747 ± 5.8

mass, so that the dimensions given for the shell are actually those of the dwarfed, abortive, protoplasmic contents in this dosage group. Any dimension of the irregularly shaped larval shell in the other dosage groups as it appeared in the picture was subject to variation depending upon the orientation of the larva within the egg. The least dimension of the ellipsoidal egg membrane, however, was independent of the orientation of the egg. The decided decline in larval size with increasing dose is apparent. Even the 180 r mean differed from the control using the "t" test by an amount beyond the 0.001 level of P. In contrast, the diameter of the egg membrane showed no linear relationship to the dosage. Because of the limited range of variability in least diameter of the egg membrane within a group, all three irradiated groups differed in one direction or the other from the control by an amount at or beyond the 0.04 level of P. This difference is considered not to be related to radiation.

At 9 days the greatest dimensions of the shells of 3 larvae sampled from each group and preserved in formalin, averaged in micra: 0 r, 713; 180 r, 730; 1,000 r, 533; and 10,000 r, 200. The inhibition of growth rate observed in the photograph at one week in the 180 r group was refuted by these data at 9 days and the conclusion reached that the dose necessary to retard larval growth was more than 180 r and less than 1,000 r.

Crawling activity, as indicated by the presence of the young snails on the sides of the aquaria where they could arrive only by their own efforts, was considered to be a possible criterion of X-ray damage. The ratio of numbers on the sides to numbers on the bottoms of the

jars throughout the course of the experiment is shown graphically in Figure 6. Only the 10,000 r group was consistently depressed, but

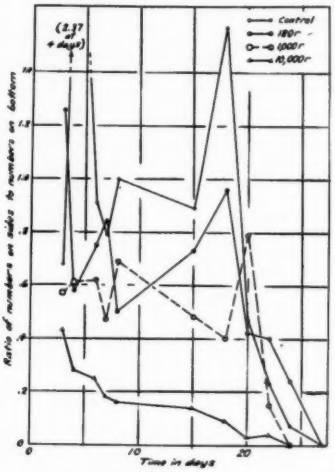

FIGURE 6

Ratio of numbers of young *Radix*-4 snails crawling on sides to numbers on bottoms of jars, as a function of time after X-raying.

the 1,000 r and 180 r groups also displayed less climbing activity during most of the experiment than did the controls.

Thais

Results of *Thais* experiments are confined to mortality studies. Although some specimens survived for more than 2 years they ate nothing with the possible exception of minute organic matter or algal scum. Eggs were laid on the glass of the aquarium but embryos were not formed. The mortalities appear in Table 3 where a tendency for mortalities to increase with dosage may be noted. *Thais*-1 was terminated by inadvertently allowing the water to become too warm early in the second month. In *Thais*-3 the 30 kr group showed remarkable resistance, 162 days elapsing before 50 per cent died. Figure 7 shows for *Thais*-2 and -3 dosage as a function of the time required for 50 per cent of the snails to die. The values were not compensated for control mortalities. Although the correlation coefficient, —0.635, at 6 degrees of freedom, falls a little short of statistical significance, a downward trend prevails.

TABLE 3

CUMULATIVE PERCENTAGE MORTALITY IN CONTROL AND IRRADIATED GROUPS ON THE INDICATED NUMBER OF DAYS AFTER X-RAYING *Thais*-1, -2, AND 3.

Thais-1

Dose in r	No. of specimens	Days after X-raying 22	24	25	26	36
0	45	4	9		11	18
200	9				22	
500	9	11				33
1,250	9				11	33
2,500	9				11	
5,000	6			17	33	50
10,000	2	100				

Thais-2

Dose in r	No. of specimens	Days after X-raying 6	7	8	9	11	18	20	24	374	427	487	566	672	765
0	17		6	12	18	24	35	41	47	59	65	71	75	82	100
5,900	8				25		38			50	62	87		100	
10,000	4				25		50				75			100	
20,000	1	100													

Thais-3

Dose in r	No. of specimens	Days after X-raying 33	123	162	192	223	268	321	348	460
0	16	6		13			37	81		100
10,000	8				38	50	62	75	88	100
17,000	8		13	62	88				100	
30,000	8			50	75	88		100		

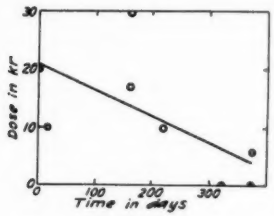

FIGURE 7
Dose of X-ray required to kill 50 per cent of *Thais*-2 and -3, as a function of time.

Amphipods

Amphipods were noted to swim well immediately following all exposures except the highest, 82 kr, when only 3 swam, and these feebly.

Weekly cumulative percentage mortalities of *Amphipod*-1 and -2 are given in Table 4. Early complete kills occurred after doses of

TABLE 4
WEEKLY CUMULATIVE PERCENTAGE MORTALITY OF *Amphipod*-1 AND -2

Experiment 1

	Dose in kilo roentgens											
Weeks	0	0	0	0	0	0	.15	.90	6.4	16	32	82
1												90
2			15	20		5			25	40	33	100
3	10	4	30	25	5	5		12	40	55	81	
4	10	4	30	30	5	5		21	40	60	86	
5	19	4	30	30	5	5	5	21	45	65	100	
6	48	4	30	35	5	5	5	21	80	85		
7	62	12	65	40	5	5	10	25	100	95		
8	71	16	85	55	19	5	19	33		100		
10	86	32	85	60	29	5	19	42				
12	86	66	85	75	38	30	24	50				
13	90	78	85	75	52	40	43	62				
14	95	86	100	80	76	55	67	79				

Experiment 2

	Dose in kilo roentgens											
Weeks	0	0	0	0	0	0	.20	.50	1.25	2.5	5.0	10.0
1		3	7			3	7	3			3	
2	7	7	13	7		3	7	7		3	7	7
3	17	17	23	17	17	100	27	27	27	21	13	30
4	50	20	30	20	27		40	43	33	31	43	53
5	70	33	40	23	33		43	67	43	48	97	97
6	87	47	50	37	57		60	77	60	59	100	100
7	97	67	70	57	67		80	93	80	66		

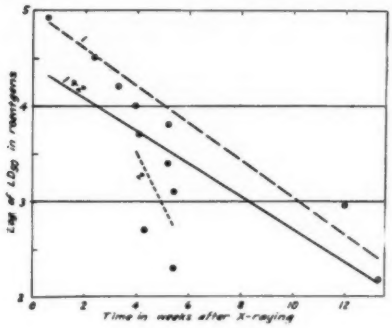

FIGURE 8

Dose required to kill 50 per cent of *Amphipod*-1 and -2 as a function of time after irradiation. Regressions of two experiments shown separately and combined.

5 kr or more. Figure 8 shows the LD_{50} as a function of time in the two experiments separately and combined. For *Amphipod*-1 the regression of $\log_{10}$ of the LD_{50} in r upon number of weeks after X-raying was: $E = 4.930 - 0.191X$. Thus, after 3 and 5 weeks respectively the LD_{50} values were 23 kr and 9 kr. The correlation coefficient, —0.983, was significant beyond the 1 per cent level of probability. For *Amphipod*-2 the relationship was not significant. For *Amphipod*-1 and -2 combined the regression was: $E = 4.416 - 0.173X$ with a significant correlation coefficient of —0.734, which for 10 degrees of freedom is beyond the 1 per cent level of probability. The combined data suggest that 50 per cent mortality would be attained in 2 weeks at 12 kr and in 8 weeks at 1 kr. These data do not include controls or correction for control mortality. Disregarding the last control group of *Amphipod*-2, which was aberrant, the average period required for 50 per cent of the controls to die was also 8 weeks, suggesting that the data are not useful for doses of 1,000 r or less.

The data of *Amphipod*-1 may be treated by the method of Irwin and Cheeseman (1939) to adjust for control mortality as shown in Table 5, yielding an LD_{50} at 3 weeks of about 16 kr and at 5 weeks of about 10 kr. When compared with the LD_{50} values of 23 kr and 9 kr given above that were uncorrected for control mortality, the corrected values are seen to decline more gradually with time. Both methods give an LD_{50} at 5 weeks of about 10 kr.

The numbers of mating pairs and of ovigerous females for the

TABLE 5

CALCULATION BY METHOD OF IRWIN AND CHEESEMAN (1939) OF LD_{50} FOR *Amphipod*-1 AT 3 AND 5 WEEKS, COMPENSATING FOR CONTROL MORTALITY
(An assumed dose of 2,400 r was interposed to fill the large gap between 900 r and 6,400 r.)

Dose			Mortality			
Actual in r	Factor	Working	Observed	Adjusted	Mean	Computation
			3 Weeks			
0			.15	0		
						4 — 1.92 = 2.08
900			.12	0		log LD_{50} = log 2,400 +
	2.7				.06	2.08 log 2.5
(2,400)		0	(.26)	.11		= 3.3802 + 0.8277
	2.7				.18	= 4.2079
6,400		1	.40	.25		LD_{50} = 16,000 r
	2.5				.32	
16,000		2	.55	.40		
	2.0				.53	
32,000		3	.81	.66		
	2.6				.83	
82,000		4	1.00	1.00		
Sum	12.5				1.92	
Mean	2.5					
			5 Weeks			
0			.15	0		
					.03	5 — 2.39 = 2.61
900		0	.21	.06		log LD_{50} = log 900 +
	2.7				.12	2.61 log 2.5
(2,400)		1	(.33)	.18		= 2.9542 + 1.0386
	2.7				.24	= 3.9928
6,400		2	.45	.30		LD_{50} = 9,800 r
	2.5				.40	
16,000		3	.65	.50		
	2.0				.68	
32,000		4	1.00	.85		
	2.6				.92	
82,000		5		1.00		
Sum	12.5				2.39	
Mean	2.5					

control and irradiated groups by weeks for *Amphipod*-1 are given in Table 6. This evidence of reproductive activity was almost lacking in groups subjected to 6,400 r or more.

Numbers of young *Amphipods* hatched after irradiation of the parents and removed in the process of counting the adults, showed a significant relationship to dosage as may be seen in Table 7 and Figure 9. Young were relatively scarce in groups exposed to 1,250 r

TABLE 6

AVERAGE WEEKLY NUMBERS OF MATING PAIRS AND OF OVIGEROUS FEMALES OF *Amphipod*-1 BY IRRADIATION GROUPS

	Dose in kilo roentgens											
Weeks	0	0	0	0	0	0	.15	.90	6.4	16	32	82
Mating pairs:												
2			1	1	2	1						
3			1	1	2	2						
4					1	2	1	1				
5	1	2		1	1	3	3					
6	1	1		1	2	1	1	2				
7	1	2		1	5	3	1	2				
Ovigerous females:												
2	2	3	1	1	1	1	2	1		1		
3		1	1	1	1	1	1	1			1	
4		2	1	1	1	1		1				
5	1	7	2	3	4	4						
6	1	2	1	1	2	2						
7		2		1	1							

TABLE 7

WEEKLY AVERAGE NUMBER OF YOUNG AMPHIPODS REMOVED DURING ADULT CENSUS IN VARIOUS IRRADIATION GROUPS FOR *Amphipod*-1 AND -2

	Dose in kilo roentgens											
Weeks	0	0	0	0	0	0	.15	.90	6.4	16	32	82
Amphipod-1:												
1	1.1	5.6	1.0	0.1	0.7	0	0	0.3	0.2	0	0	0
2	1.5	1.7	0	0	0	0	1.7	0	0	0	0	0
3	2.7	3.8	0.5	0	0.5	0.2	1.5	0	0.2	0	0	0
4	4.5	9.5	1.4	0.1	1.9	2.0	3.5	0.1	0	0	0	0
5	4.0	4.5	0	0	9.0	0.5	0.5	0	0	0	0	0
7	0	2.0	0.5	0	2.0	1.0	0	0	0	0	0	0
8	0	2.0	1.0	0	10.0	3.0	0	0	0	0	0	0
9	0	2.0	1.0	0	7.0	2.0	0	0	0	0	0	0

Amphipod-2:

	Dose in kilo roentgens											
Weeks	0	0	0	0	0	0	.20	.50	1.25	2.50	5	10
1	0	1	0	11	8	0	0	0	0	3	2	0
2	36	10	15	6	23	25	15	25	18	7	8	10
3	37	23	23	30	26	2	24	56	8	3	9	2
4	36	10	20	20	70	0	32	12	8	1	3	0
5	100	40	30	35	100	0	40	30	20	0	0	0
6	60	30	50	80	60	0	70	30	14	1	0	0
7	100	70	60	50	100	0	60	70	15	3	0	0

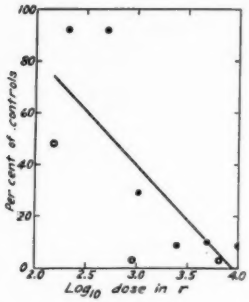

FIGURE 9

Average abundance of young amphipods for both experiments expressed as percentage of the controls, as a function of X-ray dose administered to the parents. Open circles, *Amphipod-1*; solid circles, *Amphipod-2*.

or more. A significant correlation ($P = 0.02$) exists for the following regression of abundance of young expressed as percentage of control abundance upon $\log_{10}$ of dose in r: $E = 165.5 - 42.11X$. This relationship gives about 550 r as the dose at which young were 50 per cent as abundant as in the control groups. When this dose is compared to the LD_{50} for the adults at 3 or 4 weeks after X-raying it is apparent that the production of young amphipods is from 10 to 20 times as sensitive a criterion of damage as is the LD_{50} for adults.

Artemia

Interpretations of X-ray effects on numbers and length of *Artemia* should be made in light of the fact that a period of development is involved only up to the time when the yolk is gone and availability of food and crowded conditions become the limiting factors of survival and growth. After this time the growth-inhibiting results of X-ray damage are masked by the rapid growth of the few survivors in those cultures where most larvae had died. The period from 2 to 4 days, inclusive after hatching, is practically free from this complicating situation. Table 8 gives numbers of larvae per sample for the 5 experiments. It may be noted that for the first day or two, numbers of larvae tended to be about equally reduced in the higher dosage groups in both dry and wet series. The dose at which counts were 50 per cent of control counts (LD_{50}) in this early period varied in the different experiments from about 40 kr to 80 kr. In the period from 3 to 5 days the peak in numbers and the condition of least susceptibility to X-ray

TABLE 8

NUMBERS OF *Artemia* LARVAE PER SAMPLE BY DAYS AFTER EXPOSURE SUBJECTED AS DRY OR WET EGGS TO VARIOUS AMOUNTS OF X-RAY IN FIVE EXPERIMENTS

Most values represent a single count; some are averages of two or three counts. Asterisk denotes approximate position between doses of LD_{50} for the day.

Experiment 1:

Days after X-raying	Vol. sample cc.	Dose in kr to the dry eggs 0	1.5	100
2	36	34	30 *	13
4	36	78	62 *	21
5	36	42	38 *	10
6	36	34	34 *	2
11	36	8	20	1
19	36	0	1	0

Experiment 2:

		Dose in kr to the dry eggs 0	.008	.08	.8	8	80	Dose to eggs kept wet 25 hours 0	.008	.08	.8	8	80
1	36	0	0	0	0	0	0	22	20	21	26	30 *	6
2	36	29	32	18	10	27 *	8	52	45	44	56	62 *	16
3	36	46	73	46	45	54	30*	70	77	64	82	84 *	28
4	36	54	71	63	54	75	35*	77	79	74	80	113 *	19
5	36	47	69	63	53	58	28*	51	60	69	89	77 *	4
6	36	36	51	35	38	57 *	16	69	41	53	53	59 *	1
7	36	27	29	29	25	50 *	6	43	39	41	27	35 *	1
8	36	17	22	27	20	27 *	4	39	25	26	22	20 *	0
11	36	11	10	5	6 *	2	0	19	6	12	8	4 *	0
15	36	8	2	0	6 *	2	0	6	2	6	0	4 *	0

Experiment 3:

		Dose in kr to the dry eggs 0	0	10	31	Dose to eggs kept wet 15 hours 0	3.1	10	31	Dose to eggs kept wet 24 hours 0	3.1	10	31
1	2.8	48	41	32	16	63	75	54	54	7	8	3	3
2	2.8	66	49	66	69	65	72	65	47	57	79	67	49
3	2.8	55	68	64	60	59	69	43	75	66	68	58	83
4	2.8	57	60	68	72	60	40	58	49	64	72	68	63
5	2.8	39	49	41	61	14	3	40	41	52	68	71	50
6	2.8	9	12	15	18	4	5	5	3	13	4	11	16
8	36	5	1	2 *	1	4	1	1	3	3	2	8	2
13	36	1	0	2	0	1	2	1	0	1	0	0	0

Experiment 4:

		Dose in kr to the dry eggs 0	15.1	20.8	29.8	71.8-44.6 (58)	Dose to eggs kept wet 15 hours 0	15.1	20.8	29.8	44.6	71.8
2	2.8	54	38	48	42	46 *	47	54	48	40	50 *	23
3	2.8	41	55	48	46	50 *	45	43	38	46 *	19	5
4	2.8	52	56	46	33 *	21	40	39	31	27 *	7	1
5	2.8	47	39	38	28 *	14	40	32	23 *	12	7	0
6	2.8	13	15	28	18 *	6	3	13	15	7 *	0	0

TABLE 8 (*continued*)

Experiment 5:		Dose in kr to the dry eggs						Dose to eggs kept wet 9 hours					
		0	18.8	26	38	57	93	0	12.1	18.2	28	46	80
2	2.8	28	24	24 *	12	6	3	26	16	16	15 *	10	3
3	2.8	54	58	51	46	40	43*	44	44	56	48	47	28*
4	2.8	44	36	57	51	52	44*	49	51	54	49	55	32*
5	2.8	50	44	42	48	42	28*	46	46	54	46	31 *	12
6	2.8	40	36	50	36	32 *	6	52	48	34 *	21	8	2
7	2.8	25	28	42	21 *	11	2	48	45	30 *	9	1	0
8	2.8	18	6	28	12 *	6	2	28	25	16 *	5	0	0
9	2.8	4	7	17	8 *	2	1	9	8	5 *	0	0	0
11	35	45	41	61	28 *	13	2	32	31	43 *	5	0	0
16	35	20	11	13	15 *	1	0	28	13	15 *	0	0	0
17	35	12	14	12	11 *	0	0	23	8 *	11	0	0	0
21	35	13	0	2	7 *	0	0	14	14 *	1	0	0	0

were apparent. The LD_{50} for the wet series approximated 80 kr, and for the dry series exceeded 93 kr. About the 6th day the LD_{50} declined to a level of approximately 50 kr in the dry series and to about 20 kr in the wet series. These levels were maintained for a relatively long period of a week or two, especially in *Artemia*-5 until dying off of the colonies obscured the picture. Thus, soaking the eggs in water before irradiation markedly increased their susceptibility to X-ray-induced mortality as late as 2 weeks after X-raying.

From Table 9 lengths of *Artemia* upon hatching (1-2 days) are seen to have been adversely affected in the high dosage groups. The growth-retarding effect of radiation increased to a maximum at about 4 days, when the heavily irradiated groups were outstripped by the others. Doses of 30 kr or more were accompanied by a retardation of increase in length evident at 4 days. The retardation was more pronounced in the wet series than in the corresponding dry series. The relationship between length and dose appeared strikingly in the 4-day samples of *Artemia*-5 (Figure 10) where the retarding effect on growth was nearly twice as great in the wet series as in the dry.

To utilize all data concerning relative growth-inhibiting effects of dosage in a comparison of dry and wet series, the data of Table 9 were plotted and straight lines similar to the regression lines of Figure 10 were drawn by inspection. The slopes of these lines (corresponding to the regression coefficients) were scaled from the graphs and averaged for each of the first 5 days for comparison of dry and wet series. Ratios of the slopes of wet to dry series for the first 5 days were, respectively: 0.8, 2.0, 1.6, 1.8, and 1.8. Thus, at one day of age the dry series showed a slightly and probably insignificantly

TABLE 9

MEAN LENGTH IN MICRA ON THE INDICATED NUMBER OF DAYS AFTER X-RAYING OF *Artemia* LARVAE OF VARIOUS IRRADIATION GROUPS IN FIVE EXPERIMENTS

Most means are based on from 20 to 30 measurement; for a few the range of numbers extends from 12 to 70; and starred values, on only 5 to 11.

Experiment 1:

Days after X-raying	Length in micra: Dose in kr to the dry eggs		
	0	1.5	100
2	437	437	382
5	623	557	426*

Experiment 2:

	Dose in kr to the dry eggs						Dose to eggs kept wet 25 hours					
	0	.008	.08	.8	8	80	0	.008	.08	.8	8	80
1							417	424	421	419	404	384*
2	431	434	419	427*	424	396*	507	469	492	482	459	403
3	526	533	523	490	528	421	558	579	551	592	549	439
4	587	582	579	561	553	487	606	584	591	596	596	437
5	591	574	584	571	602	500	635	639	647	647	637	
6	632	617	612	627	609	510	663	647	650	655	620	
7	721	690	712	756	674	462*	732	726	703	704	698	
8	804	680	769	724	701		831	772	776	772	736	
11	908*	811*	874*	860*			848	926*	851	884*		

Experiment 3:

	Dose in kr to the dry eggs				Dose to eggs kept wet 15 hours				Dose to eggs kept wet 24 hours			
	0	0	10	31	0	3.1	10	31	0	3.1	10	31
1	448	446	436	420	444	442	460	444	428*	422*		
2	574	593	565	564	616	588	568	525	585	582	563	519
3	654	647	640	615	637	640	629	589		621	611	568
4	699	692	672	660	722	676	692	658	673	666	670	626
5	700	704	689	695	712	732	702	700	709	705	693	690
6	679*	664	687	707			687*		712		691	684
8	706*										774*	

Experiment 4:

	Dose in kr to the dry eggs					Dose to eggs kept wet 15 hours					
	0	15.1	20.8	29.8	71.8-44.6 (58)	0	15.1	20.8	29.8	44.6	71.8
2	597	550	557	549	516	606	572	557	549	495	441
3	610	609	610	562	562	624	617	589	591	551	
4	653	631	652	646	618	666	643	651	627	595*	
5	649	661	656	661	627	673	661	644	665	654*	
6	659	673	657	652	633		670	695	665*		

Experiment 5:

	Dose in kr to the dry eggs						Dose to eggs kept wet 9 hours					
	0	18.8	26	38	57	93	0	12.1	18.2	28	46	80
4	580	543	544	510	505	474	558	534	519	511	453	393
5	611	615	600	576	559	508	628	566	584	566	504	413*
7	673	667	649	633	629*		671	627	648	582*		

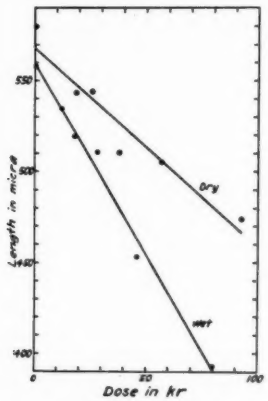

FIGURE 10

Length as a function of dose in *Artemia*-5 larvae 4 days old. Upper line from eggs X-rayed dry; lower line from eggs X-rayed after soaking 9 hours in sea water.

greater decline of growth as evidenced by length with increasing dosage of X-ray than did the wet series. By the second day the decline was twice as great in the wet series as in the dry, and also on the third, fourth, and fifth days the wet lengths showed decidedly greater, though less than twice as much, susceptibility to X-ray induced inhibition as did the dry. It is presumed that values of P, if calculated would fall between 0.001 and 0.1, indicating high statistical significance in some comparisons of regression coefficients grading down to doubtful significance in others. For example, comparison of regression coefficients of length on dose of dry and of wet 15 hours for 3-day old larvae of *Artemia*-3, selected as probably being one of the more doubtful comparisons, gave a value of "t" at the 7 per cent level of P.

Algae

Algae irradiated with soft or hard X-rays and maintained in liquid medium underwent a characteristic decline in survival at high dosages within from 4 to 12 days, and then, presumably because of release of population pressure, the survivors proliferated even more rapidly than the controls so that they had attained approximately the level of abundance of the controls within one month. In each experiment the control value was an average based upon from 2 to 6 control tube

readings. The average control value for each day was considered to be 100 per cent, upon which were based the individual values of control and irradiated tubes. The variability of the individual control percentages about the mean of 100 per cent is expressed as standard error in Table 10 for *Algae*-15 through -27 during the 4-12 day period.

TABLE 10
DATA CONCERNING CONTROLS OF ALGAE USED IN FIGURE 11 FOR COMPARISON WITH IRRADIATED GROUPS

Alga	Number of experiments	Number of tubes	Number of observations	Mean and standard error in percent, based on number of observations
Chlorella	5	25	77	100 ± 1.3
Ankistrodesmus	2	10	25	100 ± 1.3
Chroococcus	3	13	34	100 ± 3.2

A summary of results with three of the algae using liquid media is presented in Figure 11. The percentage survival relative to the

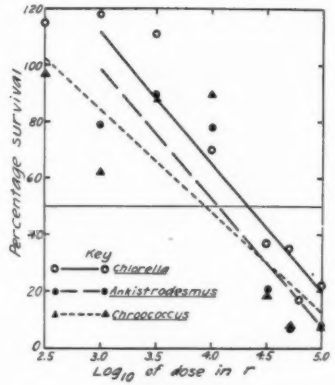

FIGURE 11
Percentage survival relative to controls for three algae in liquid medium as a function of log dose of X-ray (principally hard) administered 4 to 12 days previously.

controls is plotted as a function of the log of the X-ray dose in r units administered from 4 to 12 days previously. The correlation coefficients of survival with dose and the levels of probability for *Chlorella, Ankistrodesmus,* and *Chroococcus* respectively were: —0.95, <0.01; —0.90, 0.02; and —0.83, 0.03. The highly significant relationship for *Chlorella* might justify scaling the threshold dose at the 100 per cent survival level below which there should be no radiation-induced

mortality. This dose is seen from Figure 11 to be 1,700 r. The LD_{50} value for *Chlorella* was 22 kr; for *Ankistrodesmus,* 11 kr; and for *Chroococcus,* 9 kr. It will be noted that these values based on both soft and hard X-rays are intermediate between those to be discussed for soft and hard X-rays separately.

The relative effects of soft and hard X-rays on three algae are shown in Table 11, for which LD_{50} values were scaled from graphs

TABLE 11

COMPARISON FOR SOFT AND HARD X-RAYS OF AVERAGE MINIMUM LD_{50} VALUES IN r UNITS ATTAINED FROM FOUR TO TWELVE DAYS AFTER IRRADIATING THREE ALGAE USING LIQUID MEDIA

Alga	55 KV	200 KV
Chlorella	I. 40,000 II. 39,000	III. 20,000 IV. 10,000 V. 25,000
Chroococcus	I. 32,000 II. 25,000	III. 8,000
Synechococcus	II. > 100,000	III. > 100,000 IV. 92,000

(not presented) relating percentage survival as compared to controls, to time within the first two weeks. It is seen that for *Chlorella*, two experiments with soft X-rays gave average LD_{50} values of 40 kr, while three experiments with hard X-rays averaged 18 kr. Similarly, for *Chroococcus*, two experiments with soft X-rays averaged 28 kr and the single experiment with hard X-rays yielded an LD_{50} of about 8 kr. The first of the 4 experiments with *Synechococcus* was relatively unsatisfactory because of the wide variability among the controls. The LD_{50} was not attained in two of the other three experiments even with doses as high as 100,000 r. It is possible that higher doses should be used to attain the LD_{50} for *Synechococcus.*

In *Chlorella* 28-30, which were irradiated in liquid medium and then plated within 24 hours, doses of 31 kr were lethal to 99 per cent of the cells; 10 kr, to 50 per cent; and 3.1 kr, to almost none. The control average for the experiment was used as the basis for comparison.

Results typical of *Chlorella* 31-32 are shown in Figure 12 which is a photograph taken on the eighth day of a plate of *Chlorella*-31 colonies with ruled cellophane grid attached as used for exposing and counting. Doses in roentgens administered to the four 25-square exposed areas are superimposed on the picture. Mean counts in ex-

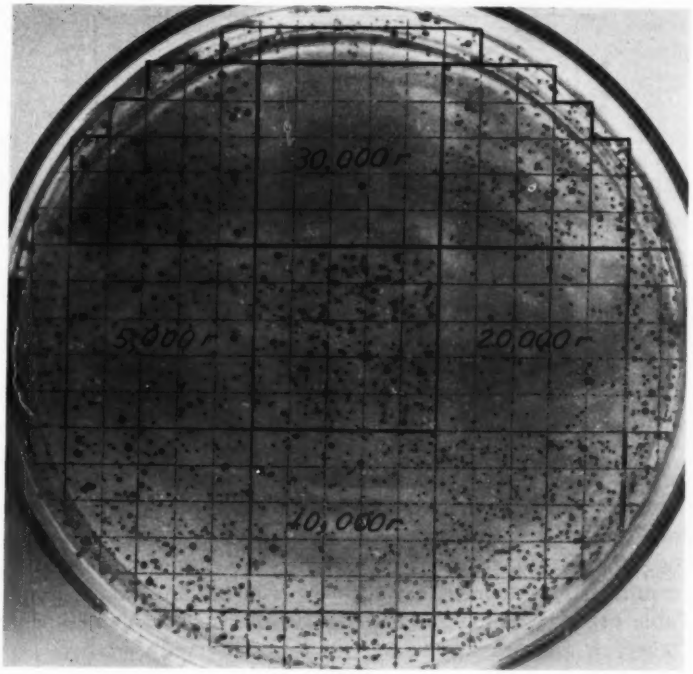

FIGURE 12

Photograph of *Chlorella* 31-32 colonies on eighth day with 5-mm.-ruled grid attached. Dosages and heavy outlines of areas used as control for the 30 kr dose were drawn on the photograph.

posed areas were converted to percentags of the mean for surrounding control areas. In heavy outline are shown the control areas used for comparison with the 30,000 r area. They consist of the 25-square central area of the plate, the 44 squares at the two sides, and the 7 squares peripherally situated from the exposed area. The colonies were too small for easy counting by the fifth day, but by the eighth day they had already become confusingly coalesced. Counts made on the seventh day did not differ significantly from those of the fifth day. Figure 13 shows for *Chlorella* 31-32, survival in exposed areas as a function of $\log_{10}$ of dose in roentgens. The regression equation for this relationship is

$$E = 3.476 - 0.765\,X.$$

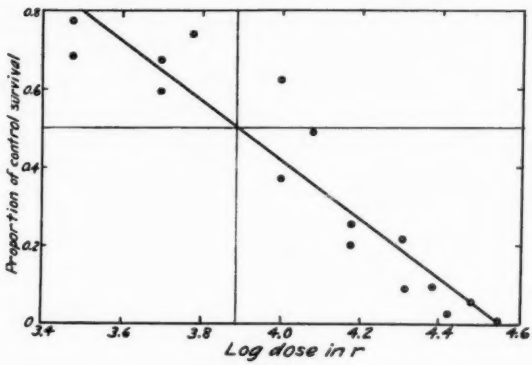

FIGURE 13

Survival of *Chlorella* 31-32 colonies in X-rayed areas in terms of percentage of survival in control areas, as a function of dose.

When E is given a value of 0.5, the LD_{50} is found to be nearly 8 kr. The correlation coefficient, —0.94 with 14 degrees of freedom has a probability value of less than 1 per cent.

For purposes of comparison of the effects in the various experiments the results of invertebrate and algal experiments are brought together in Table 12, showing the number of kiloroentgens at which 50 per cent mortality, or reduced size or activity were observed.

Discussion

Whereas with most laboratory mammals a peak mortality including most of the irradiated individuals occurs within a period usually of 30 days, little is known concerning such peaking of mortality in the case of the lower animals; the relationship of LD_{50} to time is important since low doses are effective after longer periods while high doses act quickly. Among microorganisms the choice of criterion of viability or lethality greatly affects the LD_{50} value. Standardization is difficult and perhaps not yet practical, so that except where an almost absolute criterion of viability is available as in the agar plate method, a detailed presentation of conditions and results is desirable.

In the present experiments mortalities of *Artemia*, certain *Amphipods*, and *Algae* were evaluated using control populations as the standard of comparison, but other animal mortalities were not corrected for mortalities of the controls, so that somewhat higher doses

TABLE 12

SUMMARY OF X-RAY DOSAGES IN KILOROENTGENS PRODUCING VARIOUS DAMAGING RESULTS UPON EIGHT ORGANISMS

Organism	Experiment numbers	Conditions	X-rays	Effect	Kr to produce effect: Approximate days after X-raying 5	10	20	40	80	160	320
Radix	1-3	adult	hard	LD-50*	20		12	8	2		
Radix	4	young	hard	reduced size	.2-1						
Radix	4	young	hard	reduced activity	1						
Thais	5-7	adult	hard	LD-50*	20				17	13	6
Amphipod	8-9	adult	hard	LD-50*	20-70		8-27		0.6		
Amphipod	8-9	young	hard	LD-50		0.56					
Amphipod	8	adult	hard	LD-50			16	10			
Artemia	10-14	young "dry"	hard	LD-50	93	50					
Artemia	11-14	young "wet"	hard	LD-50	80	20					
Artemia	12-14	young "wet"	hard	reduced size	10						
Chlorella	15-16	in liquid	soft	LD-50	40						
Chlorella	17-19	in liquid	hard	LD-50	18						
Ankistrodesmus	20-21	in liquid	hard	LD-50	11						
Chroococcus	22-23	in liquid	soft	LD-50	28						
Chroococcus	24	in liquid	hard	LD-50	8						
Synechococcus	25	in liquid	soft	LD-50	100						
Synechococcus	26-27	in liquid	hard	LD-50	100						
Chlorella	28-30	X-rayed liquid; plated	hard	LD-50	10						
Chlorella	31-32	X-rayed plate	hard	LD-50	8						

*Uncorrected for control mortality.

would have been required to produce 50 per cent lethality, especially in the later stages of the experiments had it been possible to maintain the control animals without mortalities. It seems unlikely that compensation for control mortality or longer survival of controls and low dosage groups would materially alter the LD_{50} in the early stages of the experiments.

Experiments involving the effects of unfiltered soft X-rays upon *Lymnaea* (*Radix*) *japonica* Jay are reported by Sonehara (1933). Dosages were given in milliampere minutes (MAM) for the eggs and in minutes, only, for adult snails. In order to arrive at an estimate of the dose in roentgens administered by Sonehara the output of the soft X-ray machine used by us on some of the algae was measured under conditions as specified by Sonehara. At 35 KV, 10 or 20 MA, and 20 cm. distance, the output was about 8 r per milliampere minute. Eggs irradiated by Sonehara with as little as 100 MAM (estimated 800 r) suffered greater mortalities in their development to the embryo stage than did the controls. Direct exposure of the eggs for 900 MAM (estimated 7,000 r) prevented formation of embryos; 1,100 MAM (estimated 9,000 r) prevented formation of veligers; and 1,300 MAM (estimated 10,000 r) prevented formation of trochophores, the earliest stage recorded. The results of *Radix*-4 where 10 kr practically prevented egg development, agree with this interpretation of Sonehara's findings.

Comparison of *Thais* with *Radix* regarding susceptibility suggests correspondence between LD_{50}'s but a longer period for the lethal effect to appear in the case of *Thais*. At 10 kr, approximately one month elapsed before 50 per cent of the *Radix* died, while in the case of the *Thais* it was approximately one-half of a year. The difference may be due to a longer life span in *Thais*. It is assumed from the annulations on the operculum that the *Thais* used were from 4 to 6 years old, while it is known from Noland and Carriker (1946) that *Lymnaea stagnalis* lives approximately one year, and Sonehara's (1933) work suggests approximately one year for *Radix japonica*.

Adult *Amphipods* exhibited roughly the same range of susceptibility as the snails, and as with other organisms the phase of the experiment concerned with the production and development of young demonstrated high susceptibility.

In the work referred to in the introduction, by Gajewskaja (1923) on *Artemia*, roentgen units were not used. Doses were from 2 to 17

minutes at 2-8 MA, "ray hardness from 3 to 6.8 after Benoit", and distance 31 cm. Kaye (1923: 109) states that a very rough approximation to the potetntial across the tube may be obtained by multiplying the "Benoist" number by a factor of from 5,000 to 10,000. Choosing an average factor of 7,500 Gajewskaja's range of potentials would have been 22-51 KV. The author states that (presumably within this range) soft X-rays were more effective than hard. Comparison with the output of soft X-ray machines at our disposal suggests that doses below 1,000 r were involved in Gajewskaja's experiments, but uncertainties would make verification of results difficult, if not impossible.

Lochhead (1941) states that thick-shelled eggs of *Artemia* are in the late blastula stage at the time of encystment. In order to ascertain the susceptibility of the early cleavage stages it would be necessary to irradiate freshly formed eggs before they were discharged from the brood pouch. After encystment the eggs become highly resistant to drought and to numerous unfavorable conditions, among them, ionizing radiations. The two week LD_{50} for dry *Artemia* eggs was about 50 kr. As would be expected, upon the resumption of embryonic development after soaking a short time in water, the susceptibility to X-ray was increased. The increase was more than two-fold under the conditions of our experiments.

The range of susceptibility of the three *Algae* upon which satisfactory experiments were performed was not great, the LD_{50} being from about 8 kr to 18 kr. The high LD_{50} obtained with *Chlorella* in liquid medium, as compared to solid, suggests an accelerated reproductive rate by the survivors in liquid media after irradiation. This would not be a factor with solid media where survival could be more precisely determined, since only viable cells could produce visible colonies. However, at high dosages in liquid media the recovery was not complete. Damage was apparently so severe at 50-100 kr that a 50 per cent retarding effect upon growth persisted one month after X-raying, at a time when lower dose groups had recovered. Unpublished portions of work done by this laboratory in 1946 on *Chlorella* from salt water showed a persistence of radiation-induced mortality as late as 4.5 months after receiving doses ranging from 100 kr to as low as 25 kr or possibly even 10 kr.

A comparison of the results on *Algae* with published material on other protista should include the careful work of Crowther (1926)

who gives as the lethal dose (LD_{50}) for *Colpidium colpoda* using soft X-rays, 60,000 e (Friedrich's) units. Quimby (1945: 691) points out that the e unit was one of the forerunners of the roentgen and is essentially the same. The resistance of *Colpidium* would therefore be slightly greater than in most of our protista. Ralston (1939) found the LD_{50} of *Dunaliella salina* to be 12,000 r when irradiated at 43 r per minute with unfiltered soft X-rays of wave-length 0.33 Angstrom units, generated at 52 KV and 10 MA from a tungsten target. The related "brown mastigophoran" studied by our own laboratory (1947) showed similar sensitivity even though the kilovoltage was 200 and the rays harder.

The greater lethality of hard X-rays than soft suggested by our experiments contradicts the qualified observation of Strangeways and Hopwood (1926: 293) that, "If doses are measured by a 'standard' air chamber, a possible increase in efficiency of less than 40 per cent in favor of long wave lengths is suggested." The contradictions existing in the literature suggest comparison of effectiveness of hard and soft X-rays as a fruitful subject for investigation. Halberstaedter and Back (1942) report for *Pandorina morum* an immediately lethal dose between 300 kr and 600 kr using soft X-rays and give the ratio of doses for delayed and immediate death as 1:100. No effects were observed below 3 or 4 kr. The high lethal dose is of a different order of magnitude from our results with non-colonial forms and might suggest a structural basis for difference were it not that Halberstaedter (1938) similarly found an LD_{50} of 350 kr for trypanosomes, which are solitary. Back and Halberstaedter (1945) found doses of from 200 kr to 700 kr necessary to stop all motion of *Paramecium caudatum* within 10-15 minutes.

Acknowledgments

Besides the acknowledgments made earlier, the writers wish to express their appreciation for assistance and helpful advice and criticism kindly given by other staff members of the Applied Fisheries Laboratory, particularly Dr. A. D. Welander, Mr. A. H. Seymour, Dr. L. R. Donaldson, Mr. Paul R. Olson, and Mr. F. G. Lowman.

Summary

Four invertebrates were subjected to graded doses of hard X-rays. The LD_{50} decreased with time. Adult snails of the genera *Radix* and *Thais* within one week after irradiation had LD_{50} values (not cor-

rected for control mortality) of about 20 kr, with considerable variability. At 40 days the LD_{50} had dropped to 8 kr for *Radix* and, after 160 days, to 13 kr for *Thais*. Young *Radix* were retarded during their first two weeks both in growth and crawling activity by doses of less than 1 kr. The LD_{50} for adult *Amphipods* at 5 weeks was 10 kr either with or without correcting for control mortality, but held more constant with time when corrected for control mortality than when not corrected. At a dose of 550 r young *Amphipods* were produced 50 per cent as abundantly as in the control groups for the entire experiment. *Artemia* eggs were X-rayed both dry and after soaking for as long as 24 hours in water to initiate development, and the young survived through the period of absorption of the yolk. LD_{50} values at 5 and 10 days respectively were: for the "dry" series, 93 kr and 50 kr; and for the soaked series, 80 and 20 kr. A reduction in growth as evidenced by body length of larvae from soaked eggs was noticeable after as little as approximately 10 kr.

Algae were irradiated with both soft and hard X-rays and were cultured in liquid and on solid media. *Chlorella* in liquid medium showed LD_{50} values within approximately one week after X-raying with soft and hard X-rays respectively of 40 kr and 18 kr. Corresponding values for *Chroococcus* were 28 kr and 8 kr, and for hard X-rays on *Ankistrodesmus*, 11 kr. When *Chlorella* in liquid medium was subjected to hard X-rays and cultured on agar plates, the one-week LD_{50} was 10 kr, and when irradiated directly on the agar plates, it was 8 kr.

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QUANTITATIVE GROWTH IN OBELIA COLONIES IN CULTURE
II. SEASON CHANGES IN RATES OF BASIC ACTIVITIES
C. Differentiation

Frederick S. Hammett

The Lankenau Hospital Research Institute, Philadelphia

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"Who is so wise as to be able to know all things?"
St. Thomas à Kempis: Imitatio Christi

Introduction

The growth activity of differentiation (cell specialization) is dominant in progression of hydranths and gonangia from cones (½s) to cylinders (¾s), and in hydranth production by stolons (Hammett, 1943). Its measure (rate) is the percentage of cones which go on; and of stolons which produce hydranths during a 24-hour time-slice (Hammett, 1950).

Rates change on season (April-September). The generally applicable factors and procedures of analysis are found in earlier reports (Hammett, 1950; 1951a, b).

The activity has 11 sources (expressions) in culture: 7 hydranth, 3 gonangial, 1 stolon. The *hydranth* are: Initially present (IP) ½s (HV); and ½s coming into being during culture from IP Buds (Bd), from buds from hydrocaulal anlagen (AN), from IP Empty hydrothecae (EM), and from Broken pedicels (BR). Also from buds from empties from IP Senile animals (SN), and buds from empties from seniles from IP Complete feeding hydranths (CO). The *gonangial* are: IP ½s (HV), ½s coming into being from IP ¼s (QT); and from ¼s from IP —¼s (MQ). Shift of stolons from stolon growth to hydranth production (ST) is the 11th expression.

All are exposed to the same season changes in environment. Season change in exogenous factors is the same for all. Differences between expressions in course of rate change are grounded in endogenous factors, not in exogenous.

The Data

Rate of each expression in each week (period) over the months (April-September) in each year is in Table 56; that for all years combined in Table 58 of the rate report (1950). The latter epitomizes the trends in the years singly; and is therefore used here (Plate 1, hydranths; Plate 2, gonangia). The 7 year average temperature over the same weeks is also given, as gotten from Table 13 of the population study (Hammett and Hammett, 1945). Period-to-period rate-temperature correlation is in Tables 1, 1a. Variability is in Table 2. The numerical designates show the order of appearance of differentiation dominance in culture, i.e., whether it is the 1st, 2nd, 3rd, 4th, or 5th activity expressed in the in-culture progression. It should be remembered that the higher the order the greater the number of in-culture antecedent steps before the activity is expressed; the greater the delay in its expression because each step takes time; the lower its rate per 24 hours because less time is available for expression of potentiality; the greater the weakening of colonies by culture conditions at time of activity expression; and the greater the sensitivity to effect of exogenous factors (1950; 1951a, b).

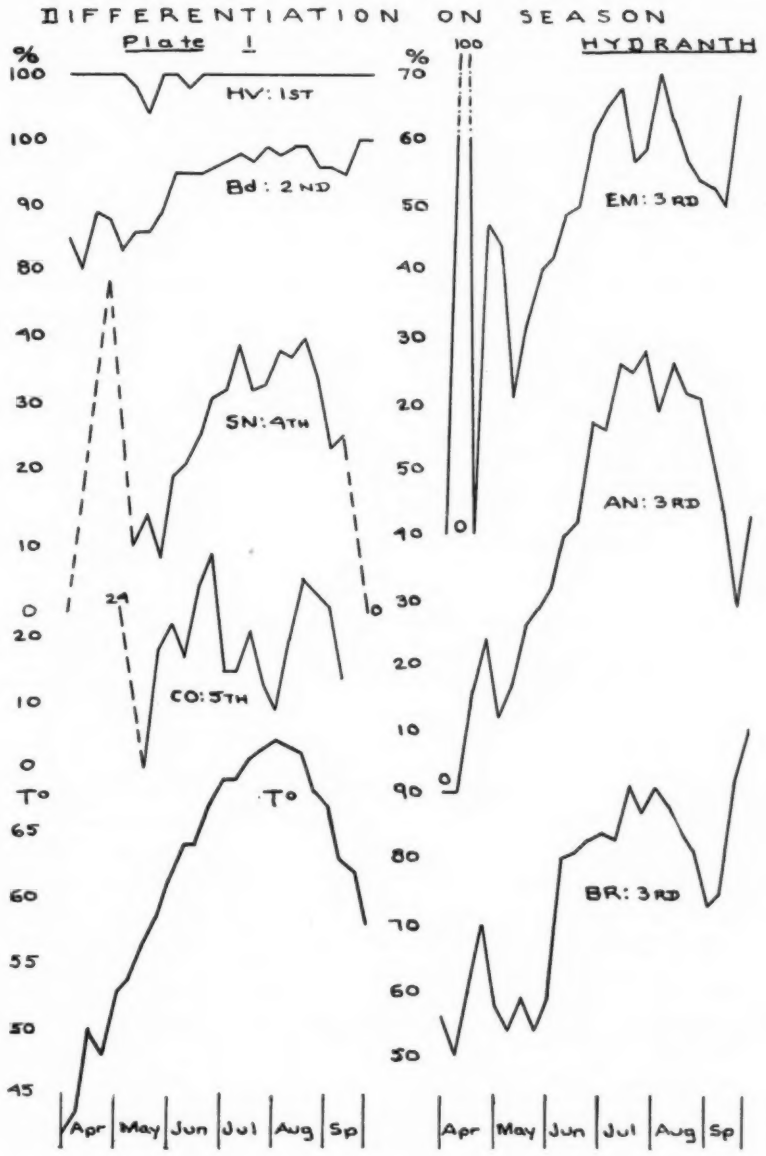
Analysis and Comment

Timing of rate changes on season differ in different years because of difference in time of release of colonies from winter inhibition, and of timing of season changes in exogenous factors; thus cancellations occur when data of all years are combined. Nonetheless the overall courses epitomize the general trends, of which three are found. (1) Rate increases to sometime in June; then tends to asymtoticism. (2) Rate increases as season advances; then falls away. (3) Rate shows no progressive change on season.

(1) This course characterizes 2nd order activity in hydranths (Bd); 1st in gonangia (HV). The course is present in all 7 years in hydranths. In gonangia the rise occurs in 5 of 6 years; the plateau in 3.

The rise accompanies temperature rise, and period-to-period changes in rate are positively correlated with those of temperature; therefore this phase is taken as temperature-conditioned. The author's interpretation is that this occurs because the time of rise is the time of most active "growingupness", which is the time of heightened sensitivity to exogenous factors (Cf. 1951b).

The levelling-off phase is attributable to colony maturing, since it


DIFFERENTIATION ON SEASON
Plate 1
HYDRANTH
%
HV: 1ST
Bd: 2ND
SN: 4TH
CO: 5TH
To
EM: 3RD
AN: 3RD
BR: 3RD
Apr
May
Jun
Jul
Aug
Sp

DIFFERENTIATION ON SEASON

Plate 2

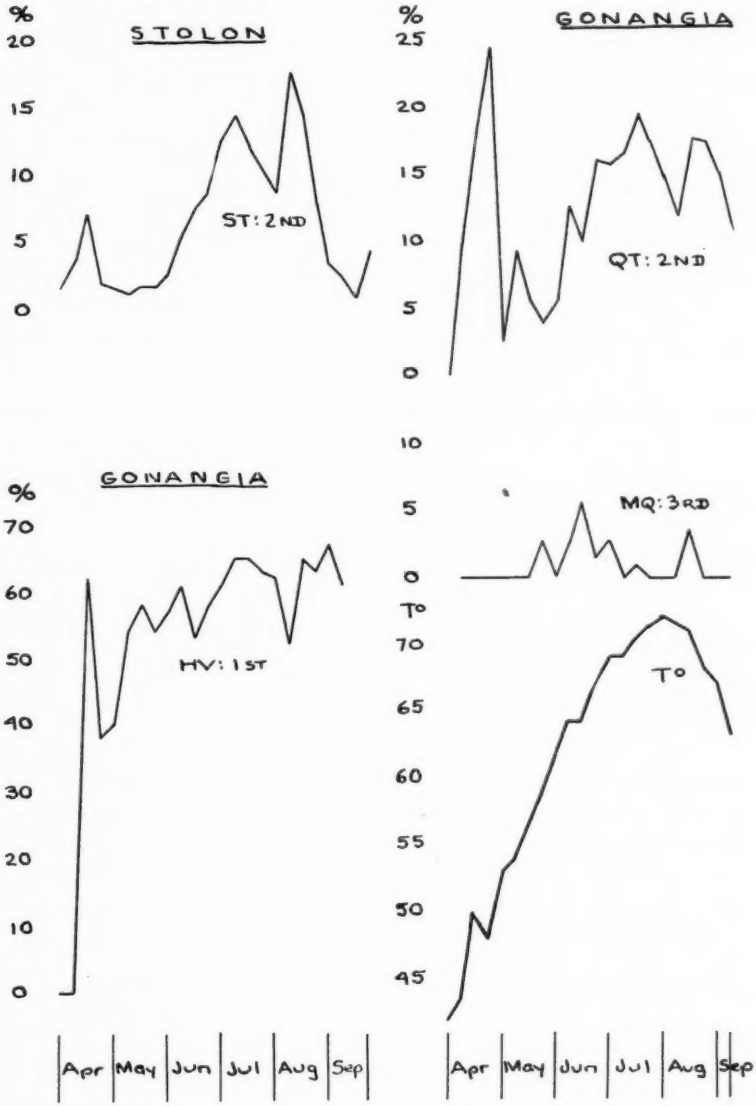


TABLE 1.—Correlation Between Rate and Temperature Change: Differentiation Hydranths

By Month

	HV (1)						Bd (2)						AN (3)						EM (3)					
	Periods			Years			Periods			Years			Periods			Years			Periods			Years		
	S	O	N	S	O	N	S	O	N	S	O	N	S	O	N	S	O	N	S	O	N	S	O	N
AP	0	0	4	0	0	2	2	2	0	1	1	0	2	1	1	1	0	1	1	0	1	1	0	1
MY	2	2	16	0	0	7	11	4	5	3	0	4	10	6	4	3	1	3	5	6	8	2	3	2
JN	0	2	26	0	1	6	16	3	9	6	1	0	14	5	9	5	0	2	15	9	4	4	1	2
JY	0	0	28	0	0	7	6	4	18	3	2	2	16	5	7	6	1	0	12	7	9	5	0	2
AG	0	0	28	0	0	7	8	5	15	4	1	2	14	5	9	5	1	1	9	10	9	3	3	1
SP	0	0	16	0	0	7	8	2	6	4	1	2	8	5	3	4	2	1	9	5	2	3	1	2
TOT	2	4	118	0	1	36	51	20	53	21	6	10	64	27	33	24	5	8	51	37	33	18	8	10

	BR (3)						SN (4)						CO (5)						ST (2)					
AP	1	3	0	0	2	0	0	0	1	0	0	1	—	—	—	—	—	—	4	0	0	2	0	0
MY	8	7	4	4	2	1	1	3	8	0	2	4	1	0	3	1	0	2	5	7	8	2	3	2
JN	16	7	5	5	2	0	12	6	9	3	2	2	7	11	16	2	4	1	20	3	5	7	0	0
JY	13	10	5	3	2	2	7	13	8	1	5	1	7	15	6	1	5	1	11	12	5	3	4	0
AG	11	9	8	2	1	4	10	8	9	3	2	2	12	7	8	3	0	4	15	5	8	4	0	3
SP	6	6	4	1	1	5	5	6	3	1	2	4	6	5	2	2	2	3	10	4	2	4	1	2
TOT	55	42	26	15	10	12	35	36	38	8	13	14	33	38	25	9	11	11	65	31	28	22	8	7

By Year

	HV (1)						Bd (2)						AN (3)						EM (3)					
33	0	0	18	0	0	5	9	3	6	3	0	2	9	5	4	3	1	1	7	5	5	3	1	1
34	0	0	15	0	0	5	6	2	7	3	1	1	5	5	5	2	3	0	8	4	3	3	0	2
35	1	3	13	0	1	4	6	2	9	3	0	2	9	2	6	5	0	0	8	3	6	2	0	3
36	0	0	17	0	0	5	9	0	8	4	0	1	9	3	5	4	0	1	5	5	7	3	2	0
37	1	1	15	0	0	5	4	4	9	1	2	2	9	3	5	3	1	1	8	4	5	3	0	2
38	0	0	19	0	0	6	8	2	9	4	1	1	12	2	5	5	0	1	10	5	4	3	1	2
39	0	0	21	0	0	6	9	7	5	3	2	1	11	7	3	2	0	4	5	11	3	1	3	2
TOT	2	4	118	0	1	36	51	20	53	21	6	10	64	27	33	24	5	8	51	37	33	18	7	12

	BR (3)						SN (4)						CO (5)						ST (2)					
33	5	7	5	1	2	2	5	2	7	1	1	3	6	5	2	2	2	0	8	6	4	2	3	0
34	10	3	2	3	0	2	2	8	4	0	3	1	5	6	3	1	1	2	7	5	3	2	1	2
35	5	7	5	1	2	2	5	5	6	1	2	2	4	4	6	2	1	2	9	3	5	3	1	1
36	4	7	6	1	2	2	4	2	8	2	2	1	5	4	4	2	2	0	9	3	5	3	0	2
37	8	5	4	3	1	1	5	7	3	1	3	1	4	7	4	1	2	2	6	6	5	2	2	1
38	9	7	3	2	2	2	10	3	4	3	0	2	6	5	4	1	0	4	11	5	3	4	1	1
39	14	6	1	4	1	1	4	9	5	0	2	4	3	7	2	0	3	0	15	3	3	6	0	0
TOT	55	42	26	15	10	12	35	36	37	8	13	14	33	38	25	9	11	11	65	31	28	22	8	7

S—Same direction of change; O—Opposite direction of change; N—No comparison.

TABLE 1a

CORRELATION BETWEEN RATE AND TEMPERATURE CHANGE: DIFFERENTIATION GONANGIA

By Month

	HV (1)						QT (2)						MQ (3)					
	Periods			Years			Periods			Years			Periods			Years		
	S	O	N	S	O	N	S	O	N	S	O	N	S	O	N	S	O	N
AP	3	0	0	2	0	0	2	2	0	1	1	0	0	0	2	0	0	2
MY	9	5	4	3	1	2	4	1	13	3	1	2	1	0	17	1	0	5
JN	13	5	6	4	1	1	15	2	7	5	0	1	2	4	18	0	2	4
JY	10	10	4	3	3	0	12	6	6	4	1	1	3	1	20	1	0	5
AG	9	7	8	3	2	1	7	8	8	3	2	1	2	0	20	1	0	5
SP	5	5	2	1	2	3	4	5	3	1	2	3	0	0	11	0	0	6
TOT	*49*	*32*	*24*	*16*	*9*	*7*	*44*	*24*	*37*	*17*	*7*	*8*	*8*	*5*	*88*	*3*	*2*	*27*

By Year

	HV (1)						QT (2)						MQ (3)					
	Periods			Months			Periods			Months			Periods			Months		
34	8	5	2	3	2	0	6	1	8	3	0	2	1	1	13	0	0	5
35	7	4	6	2	1	2	6	5	6	2	1	2	0	0	15	0	0	5
36	10	3	4	4	1	0	7	2	8	3	1	1	0	0	17	0	0	5
37	8	4	5	3	1	1	3	6	7	1	3	1	3	1	12	1	0	4
38	8	7	4	3	2	1	12	2	5	6	0	0	3	2	14	1	1	4
39	8	9	3	1	2	3	10	8	3	2	2	2	1	1	17	1	1	4
TOT	*49*	*32*	*24*	*16*	*9*	*7*	*44*	*24*	*37*	*17*	*7*	*8*	*8*	*5*	*88*	*3*	*2*	*27*

S—Same direction of change; O—Opposite direction of change; N—No comparison.

occurs simultaneously, since it is not that of temperature; and since positive correlation of rates and temperature during that time is inconstant in hydranths and virtually absent in gonangia (Cf. 1951b).

A late season transitory drop is marked in all years in hydranths, and in 5 of every 6 gonangia (shown in 4th period August of combined hydranth data, 2nd period of gonangial, Plates 1, 2). It occurs on sharply falling temperature, and coincides with shift of rate-temperature correlation to decisive positive. The drop is temperature-factored, and is attributable to colony weakening by protistic and/or algal invasion (1951a).

Culture conditions are unimportant in hydranths, since the rate is high throughout (Table 58, 1950 report), and activity is presumably expressed before culture conditions intervene (1951a). The course is clean-cut in all years. In gonangia, on the other hand, the course is not common to all years, and the rate is low, ranging from 0 to 67 (Table 58, 1950 report). Yet the overall course is like that in hydranths; therefore it is not set by culture conditions *per se*, but

TABLE 2
VARIABILITY IN EXPRESSION OF DIFFERENTIATION
(AD/M)100

Source	April	May	June	July	Aug.	Sept.
			By Month			
			Hydranths			
HV:1st	0.0	0.2	0.0	0.0	0.0	0.0
Bd:2nd	3.4	5.1	1.8	1.7	1.8	2.6
AN:3rd	100.0	51.9	23.7	19.0	14.3	24.0
EM:3rd	150.0	68.2	32.5	21.6	24.8	18.6
BR:3rd	12.1	31.6	20.2	12.4	8.6	15.2
SN:4th	133.3	146.0	67.9	43.8	58.0	66.8
CO:5th	—	154.8	86.5	72.3	64.4	66.7
			Stolons			
ST:2nd	54.4	84.2	73.5	50.6	61.9	65.0
			Gonangia			
HV:1st	41.1	33.1	18.9	24.8	20.7	16.5
QT:2nd	101.7	84.1	70.5	72.8	78.0	62.9
MQ:3rd	Indet	185.7	178.6	177.8	200.0	Indet

	1933	1934	1935	1936	1937	1938	1939
				By Year (Season)			
				Hydranths			
HV:1st	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Bd:2nd	4.0	3.4	5.0	5.4	4.0	5.0	4.7
AN:3rd	42.3	26.1	40.3	37.9	34.8	42.8	44.0
EM:3rd	23.6	24.5	32.1	52.6	34.9	41.2	47.1
BR:3rd	26.8	16.2	24.4	21.7	15.7	20.7	15.4
SN:4th	81.5	69.1	72.7	49.8	37.2	55.1	101.4
CO:5th	87.8	83.1	95.2	70.9	86.1	72.5	45.9
				Stolons			
ST:2nd	71.9	94.2	104.5	67.1	79.3	57.3	89.5
				Gonangia			
HV:1st	—	16.5	23.5	16.7	15.3	17.1	24.3
QT:2nd	—	126.7	50.0	103.7	63.7	56.3	69.9
MQ:3rd	—	175.0	Indet	Indet	125.0	142.9	200.0

Indet—Indeterminate; No progression.
Number of Variates in Table 3a.

by the endogenous factor of colony progression through immaturity to maturity.

(2) Trend of rate to increase as season advances and then decrease characterizes 3rd and 4th order activity in hydranths (AN:EM: BR:SN), 2nd in gonangia (QT) and stolons (ST). In all years. These are expressions from sources arising during culture.

The course in overall is like that of temperature (T°), and period-

TABLE 2a
NUMBER OF VARIATES (N) USED IN DIFFERENTIATION VARIABILITY COMPUTATIONS

	By Month					
	April	May	June	July	Aug.	Sept.
Hydranth						
HV:Bd:AN:ST	6	25	28	28	28	16
EM	4	24	28	28	28	16
BR	6	24	28	28	28	16
SN	3	16	27	28	27	14
CO	—	9	26	28	27	15
Gonangia						
HV	5	22	24	24	24	12
QT	6	22	24	24	23	12
MQ	4	22	24	24	22	11

	By Year						
	1933	1934	1935	1936	1937	1938	1939
Hydranth							
HV:Bd:AN:ST	19	16	18	18	18	20	22
EM	18	16	18	18	18	20	20
BR	18	16	18	18	18	20	22
SN	15	15	17	15	16	18	19
CO	14	15	15	14	16	16	13
Gonangia							
HV	—	16	18	18	18	20	21
QT	—	16	18	18	17	20	22
MQ	—	16	16	18	17	20	20

to-period changes in rates tend to positive correlation with those of temperature in all but 4th order activity in hydranths (SN). The course is taken as the product of season change in temperature, resulting from delay in in-culture appearance of activity to time when colonies are somewhat weakened by culture conditions, and sensitive to exogenous factors (1951a, b).

(3) In all years the rate shows no progressive change on season in 1st and 5th order hydranth activity (Bd:CO) or 3rd gonangia (MQ).

Season change is absent in 1st order differentiation in hydranths because rate per 24 hours is 100 per cent of potentiality in all but 4 of the 131 periods. It is never less than 99 per cent. The implication is that processes specific to the activity are innately so rapid that culture conditions and temperature effects are not operative. Endogenous control is here indomitable.

Since 5th order activity in hydranths, and 3rd order in gonangia

derive from sources emerging during culture it could be expected that the course thereof would be temperature-factored (1951a, b). But rate-temperature correlation is absent, both in course and in period-to-period changes.

In-culture appearance of these expressions is greatly delayed, because of multiple antecedent steps (hyd), or innate slow growth (gon). Time for fulfillment is much shortened (1950), as reflected in the rates (overall hyd 19.0; gon 1.0). As a result, endogenous control is feeble and colony weakening by culture conditions close to maximal. Table 2 shows that the rates of these expressions are highly variable—much more so than any other expression of the activity—which shows that the activity is here unusually reactive to the changing play of endogenous accessory and exogenous environmental factors. The indication is that the above events render rates so reactive that any trend to ordered progressive change is completely smothered. In other words absence of temperature correlation and progressive rate change is due to the unwonted variability induced by conditions incident to late in-culture appearance of activity.

The argument is backed by data of Table 3 where overall rates, number of years in which rate course is like temperature, relative extent of positive correlation of rate with temperature, and relative variability are given in order of decreasing rates of 3rd, 4th, and 5th order expressions in hydranths; 2nd and 3rd in gonangia. The seria-

TABLE 3

COMPARISON OF RATE, TEMPERATURE EFFECT, AND VARIABILITY OF 3RD, 4TH, 5TH ORDER DIFFERENTIATION IN HYDRANTHS; 2ND AND 3RD IN GONANGIA TEMPERATURE EFFECT

Order	Rate	Course				Variab
		Def	Ind	Abs	P-P	Ord
			Hydranths			
BR-3rd	79	3	2	2	1	3
EM-3rd	56	4	2	1	2	4
AN-3rd	52	5	2	0	3	5
SN-4th	32	3	0	4	0	6
CO-5th	19	0	0	7	N	8
			Gonangia			
QT-2nd	12	2	3	1	P	1
MQ-3rd	1	0	0	7	A	2
			Stolon			
ST-2nd	8	3	2	2	P	7

Def—Definite; Ind—Indicated; Abs—Absent; P-P—Period-to-Period; Ord—Order; N—Negative Trend; P—Positive Trend; A—Absent.

tions are taken from Tables 56, 58 of the 1950 report, and Tables 1-2 of this. They show that as rates (hyd) decrease, the temperature effect increases through the 3rd order, starts to decline in 4th, and expires in the 5th; that variability increases; and that shift of temperature effect from increase to decrease (4th vs. 3rd order), and from effect to no-effect (4th vs. 5th hyd; 2nd vs. 3rd gon) coincides with sharp declines in rates, sharp jumps in variability (Table 2).

To sum up: The lower the rate the later the in-culture appearance of activity; the greater the colony weakening at time of emergence of activity; the greater the variability of activity rate (1950, 1951a, b). The table thus is quantitative evidence, not only that temperature direction of rate course on season may be the product of colony weakening, but also that progression of this beyond a certain state may be responsible for absence of season change in rate because of heightened variability.

Comparison is dependable only when of the same activity, expressed in the same structure from the same immediate source. Absence of correlation in inter-activity or inter-structure is no denigration. Its presence is simply supportive and fortuitous (1950). Thus hydranth, gonangial, and stolon activity are considered separately.

The Plates show divagations from overall trends at beginning and end of season. An April-May sharp rise and fall occurs in 9 of 9 acceptable expressions, which is definite in 37, indicated in 3, absent in 19 possibilities in the years singly. A September rise occurs in 7 of 8 acceptable expressions, with rise in 37, fall in 14, and no change in 1 of the possibilities in the years singly. For obvous reasons 5th order hydranth and 3rd order gonangial are excluded from the early season count. Likewise these and the 1st order hydranth are excluded from the late-season. These events are too uniform and ubiquitous to be meaningless, but interpretation is at present impossible.

Differentiation Variability of each expression by month of all years combined, and by year (season) of all months combined is in Table 2, as computed from Table 56 (1950). The number of variates is in Table 2a. Wanted is the relative variability by expression, month, and year. Procedure is as usual (1951a). Relative rates are similarly gotten from Tables 36, 58 (1950).

The 8 hydranth cum stolon expressions, the 3 gonangial show the following order of decreasing variability: increasing rate:

Hydranth

By Year	CO > ST > SN > AN = EM > BR > Bd > HV
By Month	CO > SN > ST > EM > AN > BR > Bd > HV
Rate	ST < CO < SN < AN < EM < BR < Bd < HV

Gonangia

Year: Month	MQ > QT > HV
Rate	MQ < QT < HV

Variability by year is seasonal variability. Relative variability by year is essentially the same as by month, which shows that the gamut is not season-determined, because exogenous factors differ in different months. The seriation is endogenously determined, since it is essentially like that of increase in rate, and rate differentials are endogenously set (1950). The higher the rate the greater the stabilization of sub-tending processes, the less the variability. The fact that variabilities are higher, rates lower, in gonangia (order for order), supports this conclusion. (Cf. 1951a, b.)

Variability according to month is as follows:

Hydranth

Variability	MY > AP > JN > SP > JY > AG
Rate	MY < AP < SP = JN < JY < AG

Gonangia

Variability	AP > MY > AG > JY > JN > SP
Rate	AP < MY < AG < JN < SP < JY

Stolon

Variability	MY > JN > SP > AG > AP > JY
Rate	MY < SP < AP < JN < AG < JY

It is higher in immature (AP:MY) colonies, lower in mature (JY:AG:SP) as would be expected since immaturity is the time of instability and maturity a time of settling down. Inverse relation to rate is found at the extremes, a fact consistent with the postulate that the differentials are endogenously conditioned.

Variability according to year is as follows:

Hydranth

Variability	'35 > '33 > '39 > '38 > '36 > '34 > '37
Rate	'37 < '35 < '36 < '34 < '39 < '33 < '38

Gonangia

Variability	'39 > '36 > '34 > '35 > '38 > '37
Rate	'36 < '35 < '34 < '38 < '37 < '39

Stolon

Variability	'35 > '34 > '39 > '37 > '33 > '36 > '38
Rate	'33 < '39 < '34 < '38 < '36 < '35 < '37

Consistency is absent, presumably because of cancellations due to year-to-year differences in different rate-affecting factors.

Summary and Conclusions

Differentiation has 11 expressions in Obelia colonies during 24 hours in culture: 7 hydranth, 3 gonangial, 1 stolon. Three trends of rate on season (April-Sept.) are exhibited. (1) There is a two-phase course consisting of a temperature factored rise and a maturity determined plateau. Culture conditions are not involved. The course is product of the endogenous factor of colony progression through immaturity to maturity. (2) There is a two-phase course consisting of rise to peak as the season advances, followed by a fall, which is the product of season change in temperature because of delay in in-culture appearance to the time when colonies are weakened by culture conditions. (3) There is a course of no progressive change, which occurs when rate is endogenously set so high no other influence can break through, and when activity is so delayed in in-culture appearance that any trend of the consequent low rate to change on season is blanketed by the high variability resulting from culture conditions. The expressions differ in variability, which is inversely related to endogenously determined differences in rates. Variability is greater in immature colonies (AP - MY); less in mature (JY:AG:SP), and greater in gonangia than in hydranths.

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QUANTITATIVE GROWTH IN OBELIA COLONIES IN CULTURE
II. SEASON CHANGES IN RATES OF BASIC ACTIVITIES
D. ORGANIZATION

FREDERICK S. HAMMETT

The Lankenau Hospital Research Institute, Philadelphia

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"Without a guide to their meaning, facts alone, however compendious, are almost useless."

STEWART: SCIENCE, 1949, *110*, 179.

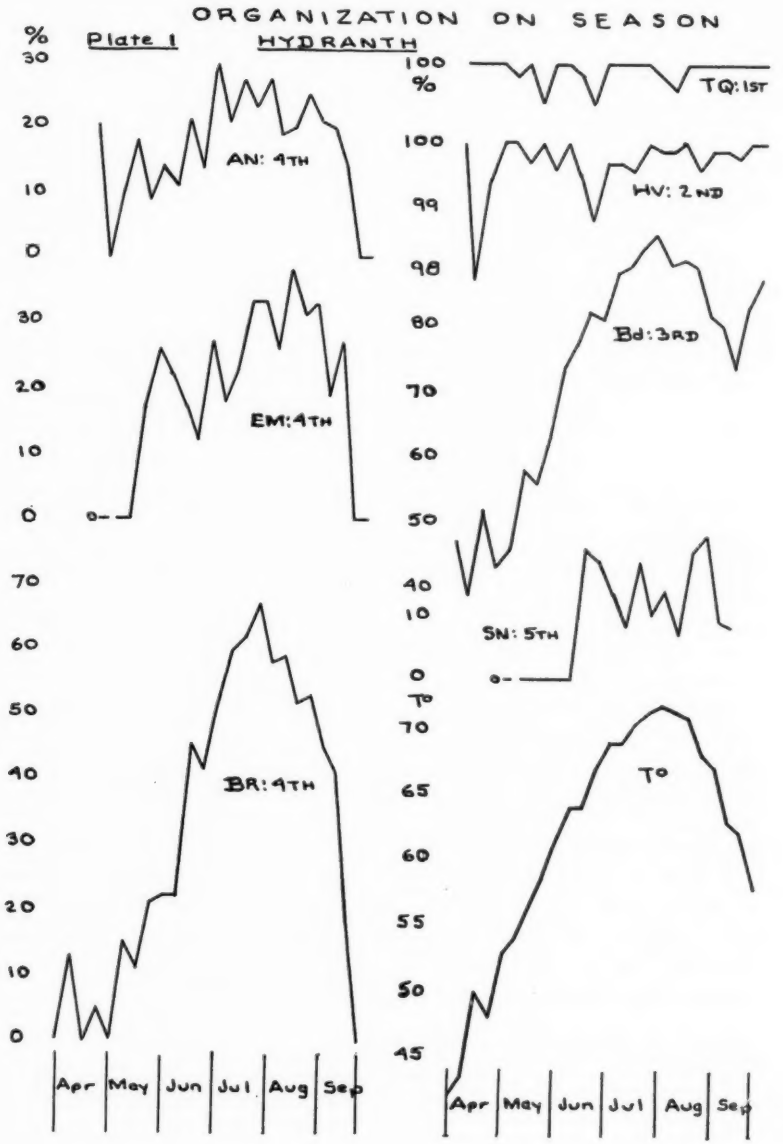
INTRODUCTION

The growth activity of organization—which is cell segregation—dominates the change of hydranths from cylinders (¾s) to be tentacled functionable animals (completes), and of gonangia from ¾s to medusae-shedding (Hammett, 1936, 1942, 1943). Its measure (rate) is the percentage of ¾s which make the change in a 24-hour time-slice. Rates change on season. The general factors and procedures of analysis are given in earlier reports (1950; 1951a, b, c).

The activity has 9 expressions in culture: 7 hydranth, 2 gonangial. Viz. *Hydranth:* progression from initially present (IP) ¾s (TQ); and those derived during culture from IP ½s (HV), from ½s from IP buds (Bd), from ½s from buds from hydrocaulal anlagen (AN), IP empties (EM), and IP brokekns (BR). And from ¾s from ½s from buds from empties from IP seniles (SN). *Gonangial:* progression from IP ¾s (TQ), and from those derived during culture from IP halves (HV). Table 1, 1950 report. All are exposed to the season change in environment. Season change in exogenous factors is the same for all. Differences between expressions in course of rate change are endogenously, not exogenously, grounded.

THE DATA

Rate of each expression in each period (week) over the months (April-Sept.) in each year is in Table 65, that for all years combined


ORGANIZATION ON SEASON
Plate 1
HYDRANTH
%
AN: 4TH
EM: 4TH
BR: 4TH
TQ: 1ST
HV: 2ND
Bd: 3RD
SN: 5TH
To
Apr
May
Jun
Jul
Aug
Sep

ORGANIZATION ON SEASON
Plate 2 GONANGIA

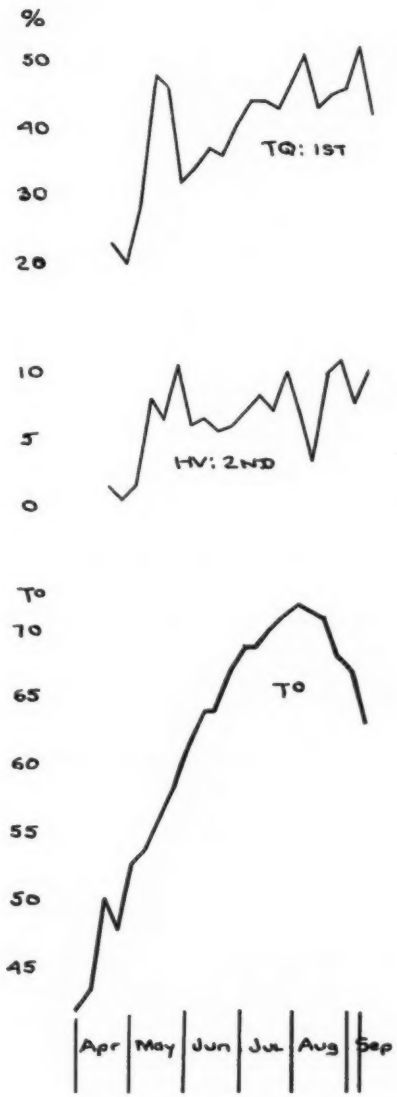


TABLE 1.—Correlation Between Rate and Temperature Change: Organization Hydranths

By Month

	TQ (1)						HV (2)						Bd (3)						AN (4)					
	Periods			Years			Periods			Years			Periods			Years			Periods			Years		
	S	O	N	S	O	N	S	O	N	S	O	N	S	O	N	S	O	N	S	O	N	S	O	N
AP	0	0	4	0	0	2	1	2	1	0	1	1	3	1	0	2	0	0	1	0	0	1	0	0
MY	1	2	17	1	2	4	2	2	16	1	1	5	9	5	6	4	1	2	7	3	8	3	2	2
JN	0	3	25	0	2	5	5	7	16	2	2	3	19	1	8	7	0	0	14	8	6	5	2	0
JY	0	1	27	0	1	6	2	3	23	1	2	4	16	3	9	5	0	2	9	11	8	2	4	1
AG	2	2	24	1	1	5	1	1	26	0	0	7	9	6	13	5	1	1	13	6	9	4	1	2
SP	0	0	16	0	0	7	1	0	15	1	0	6	9	3	4	4	1	2	7	6	3	2	2	3
TOT	*3*	*8*	*113*	*2*	*6*	*29*	*12*	*15*	*97*	*5*	*6*	*26*	*65*	*19*	*40*	*27*	*3*	*7*	*51*	*34*	*34*	*17*	*11*	*8*

	EM (4)						BR (4)						SN (5)					
AP	—	—	—	—	—	—	1	2	1	0	1	1	—	—	—	—	—	—
MY	4	1	8	3	0	3	8	3	7	4	1	2	0	0	1	0	0	1
JN	12	8	8	5	2	0	16	7	4	6	0	1	1	2	16	1	2	4
JY	17	2	9	5	0	2	20	2	6	7	0	0	6	6	15	2	2	3
AG	14	6	8	5	1	1	9	11	8	2	3	3	8	3	14	3	0	4
SP	10	2	4	5	0	2	10	3	3	4	0	3	2	2	7	1	1	4
TOT	*57*	*19*	*37*	*23*	*3*	*8*	*64*	*28*	*29*	*23*	*5*	*9*	*17*	*13*	*53*	*7*	*5*	*16*

By Year

	TQ (1)						HV (2)						Bd (3)						AN (4)					
	Periods			Months			Periods			Months			Periods			Months			Periods			Months		
33	0	0	18	0	0	5	2	1	15	1	0	4	10	2	6	5	0	0	8	3	6	3	0	2
34	0	1	14	0	1	4	2	0	13	1	0	4	7	2	6	3	1	1	5	7	3	1	3	1
35	1	0	16	1	0	4	1	2	14	0	1	4	9	2	6	4	0	1	7	4	6	2	1	2
36	0	0	18	0	0	5	0	0	17	0	0	5	9	1	7	4	0	1	3	7	6	1	4	0
37	2	2	14	1	2	2	4	5	8	2	2	1	8	3	6	2	1	2	6	5	6	3	2	0
38	0	3	16	0	2	4	2	3	14	1	2	3	10	4	5	4	0	2	11	3	4	4	0	1
39	0	2	19	0	1	5	1	4	16	0	1	5	12	5	4	5	1	0	11	5	3	3	1	2
TOT	*3*	*8*	*113*	*2*	*6*	*29*	*12*	*15*	*97*	*5*	*6*	*26*	*65*	*19*	*40*	*27*	*3*	*7*	*51*	*34*	*34*	*17*	*11*	*8*

	EM (4)						BR (4)						SN (5)					
33	7	3	7	3	1	1	8	4	4	4	1	0	3	0	6	3	0	0
34	9	3	3	3	1	1	9	3	3	3	0	2	0	0	11	0	0	4
35	8	3	6	3	0	2	8	3	6	4	0	1	1	1	9	0	0	4
36	7	0	7	4	0	0	8	4	4	3	2	0	3	1	8	2	0	2
37	10	3	4	4	0	1	12	1	4	5	0	0	3	1	11	1	0	4
38	10	1	6	3	0	2	7	7	5	2	2	2	5	5	4	1	2	1
39	6	6	4	3	1	1	12	6	3	2	0	4	2	5	4	0	3	1
TOT	*57*	*19*	*37*	*23*	*3*	*8*	*64*	*28*	*29*	*23*	*5*	*9*	*17*	*13*	*53*	*7*	*5*	*16*

S—Same direction of change; O—Opposite direction of change; N—No comparison.

TABLE 1a
CORRELATION BETWEEN RATE AND TEMPERATURE CHANGE: ORGANIZATION GONANGIA

	By Month											
	TQ (1)						HV (2)					
	Periods			Years			Periods			Years		
	S	O	N	S	O	N	S	O	N	S	O	N
AP	1	1	0	1	1	0	1	0	1	1	0	1
MY	8	6	4	2	2	2	8	2	8	2	0	4
JN	9	9	6	2	2	2	8	9	7	2	3	1
JY	8	9	7	3	2	1	8	10	6	1	3	2
AG	11	7	6	3	1	2	9	4	11	3	0	3
SP	6	4	2	2	1	3	3	6	3	0	2	4
TOT	*43*	*36*	*25*	*13*	*9*	*10*	*37*	*31*	*36*	*9*	*8*	*15*
	By Year											
	Periods			Months			Periods			Months		
34	5	6	4	1	2	2	5	5	5	1	2	2
35	6	6	5	1	2	2	6	3	8	2	0	3
36	6	7	4	2	2	1	2	2	13	0	1	4
37	8	4	5	4	0	1	5	7	5	0	2	3
38	8	5	6	3	1	2	7	9	3	2	2	2
39	10	8	1	2	2	2	12	5	2	4	1	1
TOT	*43*	*36*	*25*	*13*	*9*	*10*	*37*	*31*	*36*	*9*	*8*	*15*

S—Same direction of change; O—Opposite direction of change; N—No comparison.

in Table 67 of the rate study (1950). The latter epitomizes the trends in the years singly; and therefore is used here (Plate 1 hydranths; Plate 2 gonangial). The 7 year average temperature over the same weeks, as derived from Table 13 of the population study (1945), is also given. Period-to-period rate-temperature correlation is in Table 1, 1a, and variability in Table 2. The number of variates is shown in Table 2a. Numerical designates give the order of appearance of organization dominance in culture, i.e., whether it is the 1st, 2nd, 3rd, 4th, or 5th activity expressed in the in-culture progression. The higher the order the greater the number of antecedent steps; the greater the delay in expression of activity, the lower its rate; the greater the colony weakening by culture conditions at time of its expression, and the greater its sensitivity to exogenous factors (1950; 1951a, b, c).

ANALYSIS AND COMMENT

Three types of organization rate course on season are decipherable despite year-to-year differences in timing, onset of change in environmental factors, and considerable amplitude of period-to-period ups

S—Same direction of change; O—Opposite direction of change; N—No comparison.

TABLE 2
VARIABILITY IN EXPRESSION OF ORGANIZATION
(AD/M)100

	April	May	June	July	Aug.	Sept.
			By Month			
			Hydranths			
TQ-1st	0.0	0.2	0.2	0.0	0.0	0.0
HV-2nd	0.7	0.5	0.7	0.3	0.4	0.2
Bd-3rd	11.0	13.6	8.2	5.0	6.8	8.1
AN-4th	66.9	108.3	66.8	36.7	37.5	49.3
EM-4th	Indt	147.4	75.1	58.7	44.8	69.3
BR-4th	117.4	128.6	49.4	25.7	29.4	48.1
SN-5th	Indt	Indt	161.9	123.2	116.0	160.0
			Gonangia			
TQ-1st	56.6	51.3	48.9	36.5	32.9	38.5
HV-2nd	100.0	106.3	75.0	74.2	114.0	96.5

	1933	1934	1935	1936	1937	1938	1939
			By Year				
			Hydranths				
TQ-1st	0.0	0.2	0.0	0.0	0.2	0.0	0.2
HV-2nd	0.5	0.2	0.3	0.0	0.7	0.3	0.7
Bd-3rd	13.4	11.9	18.0	16.1	13.5	21.9	17.7
AN-4th	134.2	53.1	76.7	63.1	26.6	28.8	55.6
EM-4th	78.4	57.0	55.3	63.5	70.6	88.0	86.6
BR-4th	60.2	47.5	70.0	48.9	47.7	67.0	61.0
SN-5th	139.8	Indt	166.0	122.7	175.0	83.4	113.6
			Gonangia				
TQ-1st	—	35.9	35.3	40.9	18.4	17.7	40.6
HV-2nd	—	104.2	105.5	167.9	65.0	55.3	51.1

Indt—Indeterminate because of no progression.

and downs in rates. These are: (1) No progressive change in rate. (2) Rate rise as season advances, followed by decline. (3) No consistent change in rate.

(1) No progressive change on season is found in 1st and 2nd order activity in the hydranths (TQ:HV), in all years. Here rate is uniformly 100 per cent of potentiality, or close thereto. The implication is that processes specific to organization are innately so rapid that other factors have no chance to intrude. Endogenous control is here indomitable (1951c).

(2) Trend of rate to increase as season advances, and then decline is common to 3rd (Bd) and 4th (AN:EM:BR) order activity in hydranths; and 1st in gonangial (TQ) in all years except 1933—AN-hyd, 1934—TQ-gon. It is not well traced in the overall course of the

TABLE 2a
THE NUMBER (N) OF VARIATES USED IN ORGANIZATION VARIABILITY COMPUTATIONS

	By Months					
	April	May	June	July	Aug.	Sept.
Hydranth						
TQ:HV:BD	6	25	28	28	28	16
AN	3	23	28	28	28	16
EM	1	19	28	28	28	16
BR	6	23	27	28	28	16
SN	1	5	21	27	25	10
Gonangia						
TQ:HV	4	22	24	24	24	12

	By Years						
	1933	1934	1935	1936	1937	1938	1939
Hydranth							
TQ:HV:Bd	19	16	18	18	18	20	22
AN	18	16	18	17	18	19	20
EM	18	16	18	15	18	18	17
BR	17	16	18	17	18	20	22
SN	10	12	12	13	16	15	11
Gonangia							
TQ:HV	—	16	18	18	18	20	20

latter because differences in timing of period-to-period ups and downs and the considerable amplitude thereof in the single years result in cancellations when the data of all years combined are used. Nonetheless the course here, as in the other expressions in the class, is similar to that of temperature. And the period-to-period changes in rates tend to be positively correlated with those of temperature. So the course is taken to be the product of season change in temperature, because of delay in in-culture appearance of the activity (gonangial rate is quite low—41.3 in overall), to time when colonies are somewhat weakened by culture conditions, and therefore opened to reaction to exogenous factors (1951a, b, c).

(3) The data of all years combined of 5th order hydranth (SN) and 2nd order gonangial (HV) activity give a picture of early season rise followed by an irregular plateau. This would be interpreted as due to temperature factoring during growingupness followed by that of colony maturing (1951b, c), were it not for the fact that no consistent evidence therefor is found in the years singly. Positive correlation in period-to-period changes in rates and temperature in early season is far from general, and the course as such is far from common.

It is completely absent from the hydranth data, where rise followed by decline occurs in 3 of the 7 years; and no progressive change at all in 4. It occurs in but 2 of 6 years of gonangial record, where rise followed by decline shows in 1 year, and no progressive change in 3. In other words 5th order activity in hydranths, and 2nd in gonangia show no consistent trend of change on season.

The in-culture emergence of these expressions is considerably delayed, because of multiple antecedent steps (hyd) or innate slow growth (gon). Time for completion is short, as shown by the rates per 24 hours (overall hyd 12.1; gon 7.1). Thus endogenous control is weak; colony weakening by culture conditions maximal (1950; 1951a, b). Table 2 shows that the variability of these rates is high—much higher than that of any other, month-by-month, and year-by-year. My conclusion is that the absence of consistent change in these rates on season is due to the unwonted variability induced by conditions incident to late in-culture appearance of activity (1951c).

Organization Variability of each expression by month of all years combined, and by year (season) of all months combined, is in Table 2, as computed from Table 65 (1950). The number of variates is in Table 2a. The procedure for deriving the relative variability by expression, month, and year is as usual (1951a). Relative rates are similarly gotten from Tables 36, 67 (1950).

The 7 hydranth and 2 gonangial expressions show the following order of decreasing variability; increasing rate.

Hydranth

By Year	SN > EM > AN > BR > Bd > HV > TQ
By Month	SN > EM > AN > BR > Bd > HV > TQ
Rate	SN < AN < EM < BR < Bd < HV < TQ

Gonangia

By Year	HV > TQ
By Month	HV > TQ
Rate	HV < TQ

Variability by year is season variability. Relative variability by year is the same as by month, which shows the gamut is not season-induced, since exogenous factors differ in kind and intensity in different monthts. The seriation is endogenously determined. It is like that of increase in rate. Late differentials are endogenously set (1950).

The higher the rate the greater the stabilization of activity, the less the variability. In support of this we note that rates are lower, variabilities higher, in gonangia (order for order). (Cf. 1951a, b, c.)

Variability according to month is as follows:

Hydranth

Variability	MY > AP > JN > SP > AG > JY
Rate	AP < MY < JN < SP < AG < JY

Gonangia

Variability	MY = AP > AG > JN = SP > JY
Rate	AP < JN < MY < JY < AG < SP

It is higher in immature (AP:MY) colonies, lower in mature (JY:AG:SP), as is natural. Since immaturity is time of instability, maturity a time of settling down (1950; 1951a, b, c). Inverse relation to rate is found or indicated at the extremes. Which is consistent with postulate the differentials are endogenously conditioned.

Variability according to year is as follows:

Hydranth

Variability	'39 > '35 > '33 > '38 > '37 > '36 > '34
Rate	'37 < '35 < '36 < '34 < '38 < '39 < '33

Gonangia

Variability	'36 > '35 = '34 > '39 > '37 > '38
Rate	'36 < '35 < '34 < '37 < '38 < '39

Inverse relation between variabilities and rates is disrupted in the hydranths presumably because of year-to-year differences in rate-affecting factors. It is not so disrupted in the gonangia. It may be that when I come to compare the several basic activities some solution of the inconsistencies may emerge.

Summary and Conclusions

Organization has 9 expressions in Obelia colonies during culture: 7 hydranth, 2 gonangial. Three trends of rate on season (April-Sept.) appear. (1) No progressive change, which occurs when rate is endogenously set so high no other factor can intrude. (2) A rise to peak as season advances, followed by a decline,—a product of season change

in temperature, because of delay in in-culture appearance of activity to time when colonies are weakened by culture conditions. (3) No consistent change on season. This is attributable to unwonted variability induced by conditions incident to late in-culture appearance of activity. Variability is greater in immature (AP-MY) colonies; less in mature (JY:AG:SP), and greater in gonangia than in hydranths.

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BOOK REVIEW

Time's Arrow and Evolution, by Harold F. Blum. Princeton University Press, Princeton, N. J., 210 pages, 1951.

REVIEWED BY J. PERCY MOORE

The author's purpose in writing this book is to examine whether there may not be "strictly physical factors that have permitted no exercise of natural selection," or that may "set limits within which the latter might act." He believes that one such factor of universal applicability is the second law of thermodynamics which determines the direction of real processes. His success in marshaling fundamental data of physics and chemistry bearing upon this problem is so notable that he is assured of a wide circle of readers and of the critical discussion that he obviously desires. There is presented an epitome of the principal steps in the long journey from the formation of the primary cosmic energy-matter to the building of the complex protein molecules which are the fundamental building units of living systems. Nowhere in this long history, ticked off in billions of years, is there proof that energy has been destroyed or created. Always, if the system be visualized in all its aspects and connections, with its entropy, free energy and other energy changes, the books will balance. In the course of the argument the author freely admits making certain assumptions, but he brings in support the latest data of astronomy, geology, physics, chemistry and biology. How very modern it is the bibliography shows: of the 114 titles, 89 are dated 1940 or later, and of the remaining 25 most fall in the 1930's.

Among reviewers there will be biologists trained in modern biophysics and bio-chemistry and familiar with the mathematics of thermodynamics and quantum mechanics; but perhaps there may be permitted a few comments by an old-time naturalist who learned his chemistry and physics in the '80's when the atom was the ultimate particle and matter and energy were distinct and separate. Dr. Blum's chief reason for associating evolution with the second law of thermodynamics is that both are irreversible. This seems to be justified to the level of protein molecules. The argument is not carried to the actual course of the evolution of plants and animals as de-

scribed in such works as Gregory's *Evolution Emerging*. As described by Dr. Blum evolution followed a straight and very narrow path up to the threshold of life. But when these same protein molecules became organized into living systems the product became extraordinarily versatile and expansive. Among animals, while it is broadly true that the main line has never been reversed there are many cases of abrupt change of direction and of simplification and loss of parts which amount to reversion, especially among parasites and animals of sedentary habit.

There are many minor physical and mechanical stresses that affect the morphological characters of animals and are probably independent of both natural selection and Time's Arrow, among them the straight line hinges between the valves of pelecypod molluscs, the mutually formed articulations between two bones and the mutual limitations imposed by the growth of abutting parts.

Notwithstanding the author's disavowal, the very orderly manner in which the story is unrolled, with each step in evolution clearly dependent on what has preceded, is sure to arouse in some minds an implication of teleology. Perhaps the best way to avoid this is to read the first ten chapters in reverse. In fact, understanding of evolution above the life level in relation to inorganic evolution as portrayed by the author will become clearer if the book is read both backwards and forwards. The author's confessions regarding the genesis of the work suggest that he did just this and in fact may have begun in the middle and read both ways.

The book is so replete with facts and suggestions, all of which are essential to the argument, that the best that can be offered in the space limitation of this review is the advice to read it thoroughly. Any biologist will benefit from the author's sound scholarship and balanced judgment.